Department of Environmental Biology

Phenology and Growth of the Grasstree *Xanthorrhoea preissii* in Relation to Fire and Season

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DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

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Abstract

Australian grasstrees are a long-lived group of arborescent, monocotyledonous plants that persist in fire-prone landscapes. Renowned for their capacity to survive fire, and flower soon after, these species have long attracted the attention of biologists. A southwestern Australian species, *Xanthorrhoea preissii,* has been the subject of several recent studies, including use and verification of the "leafbase banding technique" that prompted my study. This technique, which is used to determine the age and fire history of grasstrees, correlates alternating brown- and cream-coloured, transverse bands along the stem of grasstrees with seasonal growth, and intermittent black bands with the burning of the plant. Combined, this information provides a chronology for the fire events. Fundamental to this interpretation is the assumption that grasstrees grow continually, and this growth varies annually in accordance with changes between the two contrasting seasons of its mediterranean climate. I studied *X. preissii* in two habitats (jarrah forest and banksia woodland) adjacent to the Perth metropolitan area, in southwestern Australia, focussing on leaf growth and phenology in relation to four factors important to the species lifecycle; climate, fire, reproduction and herbivory.

Leaf production monitored for grasstrees in both habitats revealed continuous growth, oscillating between maximum rates (2.5–3.2 leaves/d) from late-spring (November) to autumn (April/May), to a minimum rate of as low as 0.5 leaf/d during winter. In additional support of the "leafbase banding technique", annual leaf production was not different from the number of leafbases comprising one cream and one brown band. Synchronised with leaf production, grasstree water potentials cycled annually, with predawn readings commonly measured as 0 MPa during winter–spring and were as low as –1.26 MPa during summer, but they never exceeded the turgor loss point (–1.85 to –2.18 MPa). The fast summer growth was characterised by a fluctuating pattern of leaf production, particularly in banksia woodland, where grasstrees reliably responded to >18 mm of rainfall. Twenty-four hours after 59 mm of simulated rainfall, grasstrees in banksia woodland showed a significant increase in water potential and increased leaf production by 7.5 times. Reflecting this result, rainfall was the best climatic variable for predicting banksia woodland grasstree leaf production rate during summer, whereas leaf production of jarrah forest grasstrees was most closely correlated with daylength. Substrate differences between the two habitats can explain this variation in leaf growth patterns. While water appears to have played an important role in the evolution of

this species, growth phenology suggests that *X. preissii* may have retained a mesotherm growth rhythm from the sub-tropical early Tertiary Period.

To distinguish fire-stimulated growth from the underlying growth patterns imposed by season, leaf production and starch reserves of *X. preissii* were compared between plants from unburnt sites and those burnt in spring and autumn. Immediately following fire, *X. preissii* responded with accelerated leaf production, regardless of season. Rapid leaf accumulation during the initial flush of growth was partly at the expense of starch reserves in the stem. Although this initial flush was relatively short-lived (12–32 weeks), the effect of fire on leaf production was sustained for much longer (up to 19 months). Mean maximum leaf production rate was higher for spring-burnt grasstrees (up to 6.1 leaves/d) than those burnt in autumn (up to 4.5 leaves/d), due to optimum growing conditions in late spring/early summer. Similarly, the timing of autumn burns in relation to declining temperatures with the approach of winter appeared to dictate how rapidly grasstrees resprouted. These consequences of fire season may have implications for the reproductive success of *X. preissii*, reflected in the greater mean spike mass of spring-burnt grasstrees (1.19 kg) than those burnt in autumn (0.78 kg).

Leaf and spike growth, starch reserves and the effect of restricting light to reproductive plants on spike elongation were assessed. The emergence of the spike from within the plant's apex triggers a reduction in leaf production of up to 4.6 times that of a vegetative grasstree that is sustained until seed release 4.5–5 months later. Jarrah forest grasstrees experienced the largest trade-off in leaf production (7% lower leaf production than grasstrees in banksia woodland), and produced the shortest mature inflorescences (50% of banksia woodland grasstree inflorescences), suggesting a constraint imposed by resource availability in this habitat. During the period from inflorescence elongation to seed release starch reserves were depleted. Experimentation in the banksia woodland revealed that, although the developing spike is itself photosynthetic, it is the daily production of photosynthates by the surrounding foliage that contributes most significantly to its growth. When light was prevented from reaching the leaves the starch stored within the stem was not a sufficient substitute, evidenced by a significant reduction in spike biomass of 41%.

A fire simulation experiment with a factorial design was used to assess three factors considered important for postfire grasstree leaf growth in banksia woodland: water, ash and shade. While results identified that ash and reduced shade significantly

affect leaf growth, their effects were small compared with the stimulation derived solely from leaf removal by fire, simulated in this experiment by clipping. Clipping, also used to simulate herbivory, was imposed on a series of grasstrees at different frequencies. *X. preissii* demonstrated a strong capacity to recover in both jarrah forest and banksia woodland, even after clipping every month for 16 months. Starch reserves were depleted as the result of clipping, providing a cause of the eventual deterioration of grasstree 'health' associated with chronic herbivory. The similarity of growth responses to leaf removal independent of the mechanism (eg. fire or herbivory), provided reason to question the interpretation that grasstrees are essentially adapted to fire, rather than the alternative, that they are adapted to herbivory.

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CHAPTER 1

General Introduction

1.1 The mediterranean climate of southern Australia

Prior to the separation of Australia from Antarctica the present day mediterranean region of Australia was located at latitude 60˚ S (Specht & Dettmann 1995), well below the current location of Tasmania. It is generally accepted that 65 million years before present (BP) mean temperatures were 5–10 ˚C warmer and the climate was generally more humid with wetter seasons than the present (Specht 1973, Smith 1986, Flannery 1994, Specht & Dettmann 1995, Friend *et al*. 2001). The climatic changes resulting from the separation of these continents, with Australia's northerly drift, no doubt had a major impact on the Australian flora. Some believe that the major climatic changes that took place after the breakup of the supercontinent were such that much of the Gondwanan flora did not survive (Crocker & Wood 1947). Flannery (1994) considers that the ensuing trend of global cooling was delicately balanced by Australia's gradual drift towards the tropics, acting as a great stabilising influence on the continent. He suggests that this synchronism provided a thermally stable environment over millions of years for the existing flora to diversify, gradually adapting and radiating into the expanding arid zones. Complementing climate stability, 200 million years of relative physical stability of much of southwestern Australia, due to the absence of glaciation and major mountain building and lack of extensive submergence below sea level, is thought to have provided unparalleled opportunities for the persistence of relic taxa (Hopper *et al*. 1996). The current highly sclerophyllous flora of southern Australia most likely arose from the more xeric species that were able to cope with increasing seasonal water stress (Lamont *et al*. 2002) experienced on the edge of their former Gondwanan distribution, as periods of aridity became frequent on the continent. The start of this aridity has been placed in the late Tertiary (Kemp 1981, Specht & Dettmann 1995, Hopper *et al*. 1996, Kershaw *et al*. 2002).

1.1.1 The mediterranean climate and its influence on plant growth

Today much of southern Australia is classified as having a mediterranean climate, named for its similarity with that experienced in the Mediterranean Basin of Europe. This climate-type is shared by five areas of the world between 30 and 40˚ north and south of the equator (Hobbs *et al*. 1995b), and is restricted to the western margins of continents. Both climatic and biological features have been used by various authors to classify this climate type. Aschmann (1973) defined the mediterranean climate as having a winter period where mean monthly temperature falls below 15 ˚C, and annual precipitation is within the range 275–900 mm, whereas Nahal (1981) provided a description from a biological perspective. However, a compromise using bioclimatic criteria that identify the cool, wet winter and the period of physiological drought in summer is often preferred by biologists (Blondel & Aronson 1995). Although the present climate of southern Australia's mediterranean region is more seasonal than it once was (Specht *et al*. 1981, Bowler 1982), like other mediterranean regions it does not strictly lend itself to the traditional division of the astronomic year into four distinct seasons: spring, summer, autumn, winter. In southwestern Australia, where my study was conducted, it is more realistic to divide the years into a hot, dry summer season alternating with a cool, wet winter season. The current long-term (116 years) average annual rainfall for the area is 869 mm, of which 490 mm falls in the three calendar winter months (June–August), and mean daily temperature ranges from 13 to 24 ˚C per year (Perth Bureau of Meteorology, 2002, unpublished). Not surprising, as indicated by Aschmann's (1973) definition, temperature and moisture regimes of the two annual seasonal extremes are commonly considered to control phenology and growth of Australia's mediterranean flora (Specht & Rayson 1957a, Groves 1965, Beard 1984, Bell & Stephens 1984, Lamont & Bergl 1991, Hobbs *et al*. 1995a, Specht & Specht 1999).

Growth phases are confined to discrete periods of the year that offer a suitable combination of soil moisture and ambient temperature, and the duration of growth is generally dictated by a species' ability to cope with low levels of both of these factors. Smaller species, including tufted perennials and subshrubs, may commence growth during winter/spring when their limited root systems can take advantage of abundant water (Bell & Stephens 1984), but will become dormant when the topsoil dehydrates with the approach of summer. Consequently, these species must respond to lower

temperatures at the beginning of their growing season compared with the larger, spring/summer growing shrubs and trees that favour higher temperatures for growth and can attain sufficient water from lower soil horizons (Specht *et al*. 1981, Bell & Stephens 1984, Lamont & Bergl 1991, Pate *et al*. 1998). While water is widely accepted as of primary importance to plant growth in Mediterranean-type ecosystems (Miller 1983, Orshan 1983, Beard 1984, Lamont & Bergl 1991, Larcher 2000), in Australia some debate surrounds the evolutionary significance of plant temperature minima and maxima in determining growth phenology (Groves 1978, Specht *et al*. 1981, Bell & Stephens 1984, Specht & Dettmann 1995).

1.1.2 Fire in mediterranean Australia

Australia's shift to aridity and greater climatic variability was accompanied by an increase in fire frequency (Kemp 1981, Martin 1990, Kershaw *et al*. 2002). The culmination of climatic changes has equated to most of Australia being susceptible to fire, aside from the north-eastern pockets of tropical rainforest, where high relative humidity is the primary suppressant (Francis 1981) and the temperate rainforest in the south-east that is strongly influenced by the Antarctic climate pattern (Gill 1975). In mediterranean Australia, the combination of flammable, dry sclerophyllous vegetation, rapid fuel accumulation, annual summer drought, high temperatures and periods of hot dry winds encourage the frequent occurrence of fire (see Christensen *et al*. 1981 for detail). Palaeoecological studies have indicated that fire has been an important feature of mediterranean regions of Australia since at least 5,000 years BP (Dodson 2000, Dodson 2001a, Thomas *et al*. 2001). In south-eastern Australia, palynological evidence shows an increase in the diversity of fire ephemerals and a decrease in fire-sensitive species, in conjunction with increases in the frequency of carbonised particles around 4,000 years ago, suggesting rising pressure from fire (Thomas *et al*. 2001). However, abundant fine charcoal from sediment samples has indicated that fire was a feature of the Australian landscape prior to this time, during the upper Tertiary, and therefore was obviously the result of natural ignitions (predominantly lightning strikes) and not humans (Dodson 2001b), who entered Australia up to 60,000 years ago (Roberts *et al*. 1990, Flannery 1994, Hopper *et al*. 1996).

Australian Aboriginal use of fire is a topic of much controversy (Flannery 1994; Horton 2000), and to a lesser extent, the effect of European settlement on fire frequency. Several authors have alluded to an increase in fire frequency since European settlement of Australia (Gill & Groves 1981, Shea *et al*. 1981, Curtis 1998), yet a new method for reconstructing fire history points to the reverse (Ward *et al*. 2001): a trend consistent with conjecture by Burrows *et al*. (1995) of frequent, low-intensity, fires dominating the fire regime of pre-European jarrah forest in southwestern Australia.

1.1.2.1 Plant responses to fire

Fire is responsible for one of the most rapid and dramatic, physical and chemical changes that an environment can experience (see Jeffrey 1987 for a list of changes). It has the capacity to impact every level of an ecosystem, from microscopic to landscape scales (Christensen & Muller 1975, Gill 1993, Whelan 1995, Phillips *et al*. 2000). This translates into a broad array of interwoven factors potentially contributing to an equally diverse number of fire effects observed among the biotic components that persist within these environments. Intimate relationships exist between the life histories of the Australian biota and the passage of fire, reflecting the relatively long association between them.

A fundamental distinction between plant species on the basis of their response to fire is represented by their classification as either (re)sprouters or nonsprouters (seeders). This has been criticised by Humphrey (1974) as too crude, and improvements have been suggested by Gill (1981a). However, this simple scheme still holds considerable value among ecologists when discussing the flora of fire-prone environments, as evidenced by its continued use. Within the field of forestry the term ‘sprouting’ has been used exclusively to describe the process whereby a tree develops secondary replacement trunks (del Tredici 2001), but I prefer the broader definition that includes the means by which individuals of any resprouting species re-establishes after fire (Bell 2001).

The distinction between these two groups is likely to reflect a difference in evolutionary history. A long period free from fire is thought to have been important in the evolution of the nonsprouting, obligate seeding species (Keeley & Zedler 1978) during the Quaternary (Verdu 2000). In contrast, sprouting is considered an adaptive strategy to

recurring fire (Biswell 1974), and evolved sometime earlier during the Tertiary (Verdu 2000). The disparate fire frequencies to which these two plant groups have evolved poses a problem for management authorities designing prescribed burning programs for areas where both groups are represented. Other components of the fire regime, such as fire intensity and timing, can add further variability to plant-fire responses, complicating management programs and research in this area, where this variability is commonly ignored (Keeley & Bond 2001). Under a particular fire regime a species may respond in one direction; however, change just one element of that regime and the response can be quite different (Bradstock *et al*. 1998, Mcloughlin 1998, Vlok & Yeaton 2000). For example, Bell *et al*. (1987) demonstrated how two obligatory reseeding species (*Hakea obliqua* and *Beaufortia elegans*) growing 170 km north of Perth, Western Australia re-established successfully following autumn fire, yet were considerably less efficient in re-establishing after spring fire, possibly to the detriment of the species' future distribution in that area.

The variation in plant response to differing fire regimes emphasises the potential power of fire as a management tool, further supported by its long-lasting effect on a variety of natural landscapes, including heathland, woodland, forest and grassland (Gill & Groves 1981, Batek *et al*. 1999, Bowman *et al*. 2001). Suitable fire regimes can rejuvenate natural environments by maintaining long-term age structure and vegetation mosaics, promoting both species and habitat diversity (Catling & Newsome 1981, Yibarbuk *et al*. 2001). But caution must be applied, as an inappropriate fire regime may deleteriously alter an environment, preventing management objectives for wildlife and productivity (Tiedemann *et al*. 2000). Without specific, extensive research into the effect of fire, the prospect of identifying and implementing a suitable fire regime for a particular environment is remote.

An alternative approach is to guide management using information about past fire history (Cissel *et al*. 1999). The chronological length of recorded fire histories provided by early immigrants and settlers is often short or sketchy, possibly offering misleading observations (Tiedemann *et al*. 2000). And limitations of methods such as tree-ring counting and fire-scar evidence can prevent an accurate and definitive reconstruction of fire history (Burrows *et al*. 1995, Baker & Ehle 2001). However, recently Ward *et al*. (2001) described a novel method of aging grasstrees and determining their fire history,

inspired by the earlier work of Lamont and Downes (1979). As many new techniques are open to question, Ward *et al*. (2001) offer a brief validation of this technique (see below for more details) and demonstrate its application. The widespread application of the technique holds great promise for revealing the fire history that has shaped the Australian vegetation prior to European settlement, and may offer important insights to assist in decision-making regarding its future.

1.2 Grasstrees and their evolution, taxonomy and distribution

Balga, beluc, pol (pron. borl), blackboy, yacca, kangaroo tail and grasstree are synonymous with the conspicuous, often tree-like, monocotyledon species in the genus *Xanthorrhoea*, family Xanthorrhoeaceae (Herbert 1920, Nature Advisory Service 1974, Beard 1976, Gill & Ingwersen 1976, Missingham 1978, Clifford 1983, Staff 1989). The derivation of these names stem from aboriginal (nyoongar), colloquial and contemporary origins, but it is the abundance of alternative names that collectively demonstrate the long-standing awareness that the Australian human population has of this genus. This is by no means surprising given the unique and distinctive appearance of these endemic plants, and their typical close association with the more habitable regions of southern and eastern Australian bushland (excluding *X. thorntonii* from the dry interior).

Fossil evidence holds little value for revealing the evolutionary pathways of the monocotyledons, and similarities between different extant groups have been more successfully used for this purpose (Dahlgren *et al*. 1985). Although opposing views exist (eg. Burger 1981), it is widely considered that the monocotyledons derive from an early dicotyledonous ancestor. Deciding upon the origin or primitiveness of the grasstrees specific to the genus *Xanthorrhoea* is a problem far greater in magnitude. The order Asparagales, to which this genus belongs, is diverse in habit, that authors such as Dahlgren *et al*. (1985) attribute to a period of pronounced differentiation near the beginning of the Tertiary. In the other direction, convergent evolution has aided in confused our understanding of the relationships of descent between the members of this order. The thick trunk and rosette growth habit of many *Xanthorrhoea* species appear to have evolved independently in several different lines, including Dracaenaceae (*Dracaena* spp.) and Agavaceae (several genera). Even within the family

Xanthorrhoeaceae relatedness between *Xanthorrhoea* spp., *Dasypogon* spp. and *Kingia australis* has been debated (Bedford *et al*. 1986). More recently, in the light of significant new anatomical and molecular data, Rudall and Chase (1996) showed that the original family, Xanthorrhoeaceae, consisted of a polyphyletic assemblage, and proposed that the 10 genera be redistributed into three families, with *Xanthorrhoea* isolated in the monotypic family Xanthorrhoeaceae. Lowe (1961) places the family Xanthorrhoeaceae slightly below the world average advancement index for monocotyledons, but above that of the Liliaceae. The overall impression is that this is an 'old' family of flowering plants but actual evidence is lacking.

In Western Australia, *Xanthorrhoea preissii* is the most common species, although it can be mistaken for a number of other species in the genus. Adding to this potential confusion is the contentious issue of grasstree classification, both the placement of the genus at family level (Staff & Waterhouse 1981, Bedford *et al*. 1986, Rudall & Chase 1996) and the definition of species (Lee 1966). Novel approaches to resolve this issue have been tried, including the examination of the chemical constituents of resins from different grasstree species (Birch & Dahl 1974). An intensive collecting program aimed at refining the differences between a number of species variants in eastern Australia revealed that the amount of variation within recognised groups of *Xanthorrhoea* tended to blur the accepted boundaries that were used to separate them (Lee 1966). Conclusions drawn suggested that the intergradation between species of *Xanthorrhoea* partly reflected the variation associated with distribution, and hybridisation in areas where species were cohabiting. In southwestern Australia, *X. reflexa*, originally considered a separate species (Herbert 1920), is now considered synonymous with *X. preissii* (Bedford *et al*. 1986). The distribution of this species is restricted to the more mesic southwestern corner of Western Australia, which covers an area of 309,840 km^2, bounded by a line extending from Shark Bay on the north-western coastline to Point Culver in the southeast (Beard 1990). Specifically, *X. pressii* is a common component of various vegetation types including forests, woodlands and shrublands, existing on a variety of soil types from Jurien Bay to the Albany region (Corrick & Fuhrer 1996). Within this range, solitary individuals rarely occur and more often exist in groves.

1.2.1 Grasstree growth and response to fire

Much of what is currently known about grasstrees describes their response to fire, as it is during the postfire period that these plants experience their most dynamic and 'intriguing' vegetative and reproductive growth. Like other grasstrees, *Xanthorrhoea preissii* is a classic example of a species that resprouts following fire; the blackened stem is testament to its fire-resistance. Immediate survival of fire can be attributed to the insulation of all vulnerable portions of the plant, consistent with the strategy used by many palm species (McPherson & Williams 1998). A continuous, 5–9 cm thick layer of dead leafbases cemented together by resin (Birch & Dahl 1974) shields the inner living stem, and the shoot meristem is afforded protection by the densely packed, moist, young leaves at the apex. The effectiveness of this foliar insulation is not only exemplified by the survival of the meristem, but by the survival of many invertebrates that burrow deep into the crown (Whelan *et al*. 1980). After the smoke has cleared grasstrees are one of the first plants to commence recovery, resprouting rapidly from the apical meristem (Specht & Rayson 1957a, Baird 1977, Bülow-Olsen *et al*. 1982, Gill 1993).

The great success of grasstrees in surviving fire is reflected in the ages that these plants can achieve (Gill & Ingwersen 1976, Lamont & Downes 1979, Staff & Waterhouse 1981) and their success throughout their fire-prone distribution. An early estimation of age by Lewis (1955) placed a young individual of *X. australis* at +6,000 years old, which can only be considered as fanciful. A more realistic estimate of 375 years was made using radiocarbon-dating of the oldest leafbases on a single specimen of *X. preissii*, collected near Perth, Western Australia (Meagher 1974). Several plants of *X. australis* suggested a range of ages between 50 and 350 years, based on a calculated growth rate of 9 mm y^{-1} (Gill & Ingwersen 1976). The latter two results are certainly more consistent with the annual vertical growth rate of 10–20 mm estimated for *X. preissii* by Lamont and Downes (1979), using a technique that is supported by recent research (Ward *et al*. 2001).

In addition to vegetative growth, grasstrees readily flower soon after fire. Disturbance can sometimes induce flowering in plants. Unusual bursts of gregarious flowering have been attributed to cyclone damage in a number of tropical rainforest species (Hopkins & Graham 1987, Attiwill 1994). In more temperate environments the passage of fire can stimulate similar flowering events in numerous plant species (Gill 1981a, van der

Moezel *et al*. 1987). Of these, several are considered to be almost completely reliant on fire for reproduction: various orchid species (Erickson 1951, Willis 1970), *Xanthorrhoea* (Specht *et al*. 1958, Staff 1976, Baird 1977), and South African fire-lilies (Levyns 1966, Martin 1966). However, while the majority flowers profusely after fire, they also flower to a lesser extent during interfire periods (Gardner 1957, Black 1963, Martin 1966, Howell *et al*. 1972, Baird 1977, van der Moezel *et al*. 1987). The long magnificent grasstree inflorescence is similar among many of the species in this genus (*X. australis*, Specht *et al*. 1958, *X. johnsonii*, Bülow-Olsen *et al*. 1982, *X. fulva*, Taylor *et al*. 1998). In *X. preissii*, this consists of a smooth green peduncle, up to 1 m long, called the scape, that supports the flower-bearing rachis (spike), 1–2 m long.

Not surprisingly, the spectacular flowering display initiated by a fire appears to have attracted the most attention from biologists studying grasstrees (see references above in addition to Lewis 1955, Gill & Ingwersen 1976, Staff 1976, Lamont & Downes 1979, Gill 1993, Curtis 1998, Ward & Lamont 2000), yet no information exists on the impact of this substantial reproductive effort on the individual. Similarly, while postfire growth has been repeatedly observed and documented as rapid, for a number of grasstree species, little detail is available about this response, leaving many questions unanswered: how does this response compare with growth of unburnt plants? What is the duration of the enhanced growth? How is postfire vegetative growth affected by subsequent reproduction?

1.3 The leafbase banding technique

The leafbase banding technique, as it is known, derives its name from the patterning on the grasstree stem, visible after the removal of surface charring caused by fire (Ward 1996, Ward & Sneeuwjagt 1999, Ward *et al*. 2001. Fig. 1.1). Comprised of persistent dead leafbases, the surface of the grasstree stem shows a series of alternating, cream and brown, parallel bands that traverse the longitudinal axis of the grasstree stem. Pairs of the cream and brown bands have been equated with annual growth increments and offer a chronological sequence from the base to the crown of the plant, in much the same way as provided by the concentric growth rings of some trees. In addition to the cream and brown bands, intermittent black bands are located along the stem, marking when the oldest living leaves on the plant were burnt during fire (Ward *et al*. 2001). These two

pieces of information combined can provide a fire history of grasstrees that may be extrapolated to the surrounding environment.

Fig. 1.1 In this photograph of a section of grasstree stem (*Xanthorrhoea preissii*), the colour patterning of leafbases is quite pronounced, after grinding has removed the 3–5 mm layer of charcoal. The seasonal bands are visible as the 'tiger-like' cream and brown stripes perpendicular to the length of the stem, interrupted by two distinct black fire bands (photograph courtesy of B. Lamont).

1.4 Validating the leafbase banding technique

The most recent work on grasstrees concerns *X. preissii*, and has focused on the validation of the leafbase banding technique. The distinction of the bands on the basis of colour has been confirmed (Burrows 1998), and the width of each pair of bands has been shown to coincide with undulations in the grasstree stem beneath the protective covering of leafbases, previously used by Lamont and Downes (1979) to age grasstrees (Ward *et al*. 2001). Of importance, cream bands corresponded with the peaks, or the widest sections of the stem, and the brown bands corresponded with the troughs, or the narrowest sections of the stem, suggesting a possible seasonal basis for their colour difference. In keeping with this, Tomlinson and Zimmermann (1969) note the dependency of 'cambium' vigour, which contributes to anomalous secondary thickening in *Xanthorrhoea* (Staff & Waterhouse 1981), on shoot vigour. Regular fluctuations in the concentrations of nutrients in the cream and brown bands were also consistent with

expected seasonal depositional patterns (ie. high in the fast-growing season and low in the slow-growing season. Burrows 1998, Ward *et al*. 2001). Also, histological investigations attributed differences in the concentrations of tannin cells, particularly in the inner cortex, to the colour difference between the leafbases of the seasonal bands and black fire bands (Colangelo *et al*. 2002).

Despite observations of postfire growth vigour, a study of stem banding patterns revealed little evidence to support the hypothesis that wider bands would occur after fire (Eldridge 2000). In fact, the only evidence of a postfire growth flush was a trend for leafbases of the cream and brown bands produced after fire to be thicker than those produced immediately prior to fire. However, significant to the application of the technique, Eldridge's work demonstrated the consistency of band-widths along the stem of *X. preissii,* alluding to a reasonably constant growth rate over time. This information supports the use of mean band width from areas of the stem where bands are clearest to estimate time intervals between fires (black bands) from areas of the stem where the seasonal cream and brown bands are not as easily distinguishable (eg. near the base).

1.5 Purpose of my thesis

Without proper validation, information brought to light through the application of the leafbase banding technique may be treated with scepticism. Leaf production and leafbase production are clearly equivalent, and their key position as the basis for the leafbase banding technique influenced the direction taken by my study. The need to expand the knowledge base about environmental controls of grasstree leaf phenology and growth was identified as an area of grasstree biology vital to the development of an understanding of the banding phenomenon. But to avoid simply creating a mosaic of disjunct information for the sole purpose of technique validation, this study was designed to provide a more comprehensive picture of grasstree ecology to supplement the current gaps in knowledge of these 'charismatic' species, and to further our global knowledge about plant fire ecology. Under the general theme of growth-environment relations, each chapter in this thesis develops a particular area of the natural history of the grasstree *X. preissii*, providing material for a discussion of the banding technique in the final chapter.

Grasstrees persist under an array of factors dictated by their habitat and the regular cycling of a seasonal climate, which in turn influences growth, both above and below ground. Chapter 2 follows this line of investigation by examining the way climate influences grasstrees in two contrasting habitats. Inevitably, the naturally fire-prone habitats of *X. preissii* are eventually exposed to fire, the source of ignition by either lightning or humans, triggering the well-documented recovery response. Using data from unburnt grasstrees as controls, Chapter 3 examines the effect of prescribed burning on grasstree growth, comparing season of burn and habitat as factors contributing to crown regeneration. Also, in this chapter the role of reserved starch within the stem is introduced as part of an investigation into how grasstrees manage resource costs associated with growth. Chronologically, reproduction typically follows burning of grasstrees, and is likely to represent an energetically costly period for these plants. Chapter 4 considers these costs and discusses the results in relation to the timing of fire and the significance of reproductive timing. Until Chapter 5 the discussion of grasstree growth predominately focuses on the effect of abiotic elements like fire, not considering the biotic component of its environment. Specifically, *X. preissii* forms an integral part of the life history of many animals. While grasstrees play host to a wide array of invertebrates and small vertebrates, offering shelter, protection against predators and fire (Whelan *et al.* 1980), another suite of animals relies on these plants for sustenance (McNee 1997, Shepherd *et al.* 1997). This chapter has two distinct parts; (a) factors contributing to the 'fire effect' in *X. preissii*, and as alluded to, (b) the impact of herbivory on *X. preissii*. The management of these seemingly disparate topics within the same chapter was a deliberate tactic to contrast two possible environmental pressures with their evolutionary significance to *X. preissii* during post-Gondwanan times. Finally, after the implications of season, fire, reproduction and herbivory on grasstree growth are thoroughly discussed, the concluding Chapter 6 concentrates on the topic that instigated this study: validation of the leafbase banding technique. Information and interpretations developed under each chapter are drawn upon to explore the relationship between leafbase production and the formation of the distinct colour bands.

1.6 Field sites: jarrah forest and banksia woodland

X. preissii is common throughout bushland adjacent to the Perth metropolitan area of Western Australia. Because of this species' ubiquity, it was possible to replicate much of my work in two distinctly different environments, jarrah forest (Fig. 1.2a) and banksia woodland (Fig. 1.2b), broadening the application of my results. Although the two adjacent habitats share the same Mediterranean-type climate, their geology and vegetation composition differ markedly. Jarrah forest is restricted to the Darling Range, east of Perth, separated from the Swan Coastal Plain (location of banksia woodland) by the steep Darling Scarp. The Darling Range undulates with crests reaching 280–320 m and swampy valley floors some 50–100 m below (Churchward & Dimmock 1989). The sandy and gravelly, ferruginous, jarrah forest soils are formed on a lateritic mantle, that originated from deep and intensive weathering of the granite and granitic gneiss parent rock (Churchward & Dimmock 1989). Banksia woodland sites studied fall on the westerly boundary of the Bassendean Dune System on the Swan Coastal Plain (Bettenay 1984). In stark contrast to the Darling Range, this area was formed almost entirely of depositional material originating from fluviatile or aeolian activity (McArthur & Bettenay 1974), and characterised by low hills comprised of heavily leached, deep siliceous sand. Globally the soils of both of these habitats are nutritionally impoverished, due to a long history of weathering and leaching.

Although called jarrah forest (*Eucalyptus marginata*), the upper-storey canopy to a height of 30 m (Marshall 1986) often contains a considerable number of marri trees, *Corymbia calophylla* (syn. *E. calophylla*). Beneath this canopy is usually a well-defined mid-storey often including *Banksia grandis*, *Allocasuarina fraseriana* and *Persoonia longifolia*, and a dense ground cover of mainly leguminous and proteaceous woody perennial shrubs (McArthur & Bettenay 1974, Bell & Heddle 1989). Generally lacking the larger canopy tree species, banksia woodland has a more 'scrubby' appearance. *B. menziesii*, *B. attenuata*, *B. ilicifolia* and *Allocasuarina fraseriana* commonly co-dominate to a height of 10 m (Marshall 1986, Corrick & Fuhrer 1996), with a dense understorey of sclerophyllous perennials (McArthur & Bettenay 1974), dominated by *Hakea*, *Dryandra* and other Proteaceae species.

Fig. 1.2 (a) the unburnt jarrah forest site (MC) on the Darling Range, with several waist height individuals of *X. preissii* in the foreground. (b) the more open unburnt banksia woodland site (YC) on the Swan Coastal Plain, again with grasstrees visible throughout.

These gross vegetational differences are interpreted by Beard (1983, 1984) as a consequence of variability in soil moisture dictated by the holding-capacity, permeability, drainage and aeration properties of soil, rather than nutrient availability. The infiltration capacities of the jarrah forest surface soils are high, and are rarely exceeded by rainfall intensities (Schofield *et al*. 1989, Silberstein *et al*. 2001). This is coupled with a large surface soil water storage capacity, that experiences major seasonal changes due to the low permeability of the clay subsoil (Schofield *et al*. 1989). In contrast, among the banksia woodland the permeable sandy soils are heavily infiltrated during winter until saturation is reached and maintained through to September (McArthur & Bettenay 1974). With the onset of high summer evapotranspiration rates, soil moisture on the sandplain falls rapidly below field capacity, and no water is available within several metres of the surface by late summer (McArthur & Bettenay 1974). During this time water is only available beneath this depth.

Throughout this study, three jarrah forest sites near Mundaring (30 km east of Perth CBD), and six banksia woodland sites near Yanchep and Wanneroo (70 and 40 km north of Perth CBD respectively) were used (Fig. 1.3). Table 1.1 offers detailed information about each site, including site names and appropriate acronyms that are referred to throughout my thesis.

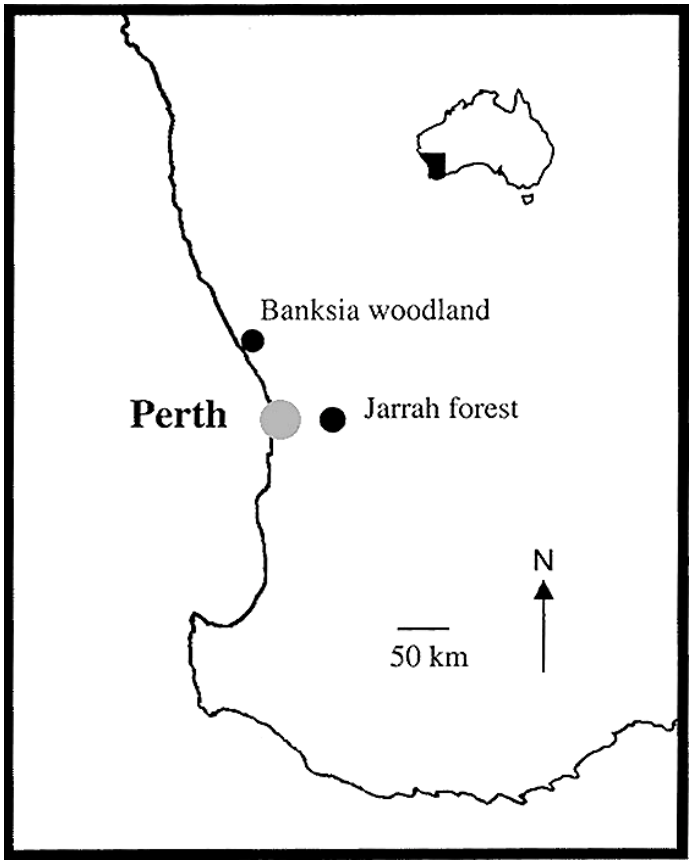

Fig. 1.3 Location of the jarrah forest and banksia woodland study sites in relation to Perth, Western Australia.

Table 1.1 List of all sites used during this study, indicating site name and acronym used in text, location, dominant vegetation type, soil type and recent fire history. Mundaring sites (jarrah forest) are located on the Darling Range and all others (banksia woodland) on the Swan Coastal Plain. Identifications were made using Marchant *et al*. (1987) and nomenclature follow Paczkowska and Chapman (2000).

Site name (acronym used in text)	Latitude longitude	To Perth: bearing, distance	Dominant flora (in order of abundance)		Soil type	Last burnt
			Overstorey	Understorey		
Mundaring control (MC)	32˚ 00' 14.6" S 116˚ 11' 10" E	102˚, 31.4 km	*Eucalyptus marginata* *Corymbia calophylla* *Banksia grandis*	*Bossiaea aquifolium* *Macrozamia riedlei*	lateritic	1988
Mundaring spring burn (MSB98)	31˚ 59' 44.2" S 116˚ 8' 17" E	102˚, 26.8 km	*Eucalyptus marginata* *Corymbia calophylla* *Banksia grandis*	*Bossiaea aquifolium* *Xanthorrhoea gracilis* *Hakea amplexicaulis*	lateritic	15 Oct 1998
Mundaring autumn burn (MAB00)	32˚ 4' 29.8" S 116˚ 5' 43" E	123˚, 25.8 km	*Eucalyptus marginata* *Corymbia calophylla* *Banksia grandis*	*Bossiaea aquifolium* *Hakea amplexicaulis* *Acacia pulchella*	lateritic	29 May 2000
Yanchep control (YC)	31˚ 26' 19.4" S 115˚ 38' 7.3" E	343˚, 61.5 km	*Banksia attenuata* *Banksia menziesii* *Banksia ilicifolia*	*Eremaea fimbriata* *Scholtzia involucrata* *Hakea ruscifolia*	leached sand	1994
Yanchep spring burn (YSB98) ★	31˚ 23' 9.2" S 115˚ 35' 54" E	342˚, 68.2 km	*Banksia attenuata*	*Hakea prostrata* *Conospermum triplinervium* *Dryandra lindleyana*	leached sand	28 Oct 1998

Table 1.1 Continued over page.

Table 1.1 Continued from over page.

Site name (acronym used in text)	Latitude longitude	To Perth: bearing, distance	Dominant flora (in order of abundance)		Soil type	Last burnt
			Overstorey	Understorey		
Wanneroo spring burn (WSB99)	31˚ 35' 56.1" S 115˚ 45' 42" E	350˚, 41.0 km	*Banksia attenuata* *Banksia menziesii* *Allocasuarina fraseriana*	*Hibbertia hypericoides* *Hakea prostrata* *Jacksonia sericea*	leached sand	4 Oct 1999
Yanchep autumn burn (YAB99)	31˚ 26' 22.9" S 115˚ 40' 57" E	347˚, 60.0 km	*Banksia attenuata* *Banksia menziesii* *Banksia grandis*	*Hibbertia hypericoides* *Stirlingia latifolia* *Petrophile macrostachya*	leached sand	22 April 1999
Yanchep autumn burn 2 (YAB00)	31˚ 25' 54.7" S 115˚ 40' 23" E	346˚, 61.1 km	*Banksia attenuata* *Banksia menziesii*	*Conospermum triplinervium* *Petrophile macrostachya* *Eremaea pauciflora*	leached sand	24 May 2000
Yanchep fire simulation experiment (YFSE) ★	31˚ 23' 9.2" S 115˚ 35' 54" E	342˚, 68.2 km	As for YSB98	As for YSB98	leached sand	c.10–15 years ago

★ these sites were adjacent to each other, separated by a 6 m wide sand track.

Note: All sites had abundant *Xanthorrhoea preissii*, and therefore this species is not included in the vegetation descriptions. For sites burnt prior to this study (October 1998) fire history information was retrieved from CALM (The Department of Conservation and Land Management) records.

CHAPTER 2

Vegetative growth phenology of *Xanthorrhoea preissii*

2.1 Introduction

Organisms invariably exhibit a natural growth rhythm reflecting their interaction with both abiotic and biotic elements of their environment. Phenology refers to the timing of recurring biological events (eg. growth phases and flowering). The challenge is to both identify and explain factors that control the events, which characterise this rhythm. Seasonality can be considered as a specific, and most common, determinant of phenological timing, where groups of biotic and abiotic events are correlated with definite periods of the calendar, or solar year (see Lieth 1974). Higher plants are well represented among the available phenological studies, with particular emphasis on agriculturally important species. Few studies have been conducted on natural vegetation (Lieth 1974), and even less have considered both the above- and below-ground components of vegetation.

In the mediterranean environments of southern Australia, vegetative growth phenology varies considerably among the different growth forms (Specht *et al.* 1981, Bell & Stephens 1984). Collectively, growth is usually restricted to a discrete portion of the year by seasonal fluctuations of essential resources, such as light, water, nutrients and temperature (Specht 1973, Specht & Brouwer 1975, Kummerow *et al.* 1981, Lamont & Bergl 1991, Hobbs *et al.* 1995a). In my study, investigation was limited to the effect of climatic variables to explain the annual growth pattern of *Xanthorrhoea preissii*. Broad yet consistent patterns of annual growth phenology have been demonstrated for the flora of Australia's Mediterranean-type regions (Specht & Rayson 1957a, Lamont 1976, Bell & Stephens 1984, Lamont & Bergl 1991). The correlation between plant size and phenology identified by Bell and Stephens (1984) during a study of kwongan (scrub-heath) vegetation in southwestern Australia is a convenient generalisation of these patterns. Winter/spring growing species are predominately tufted perennials and subshrubs, spring growing species are low shrubs, and spring/summer growers consist of the taller shrubs and small trees. These three patterns of growth conveniently correspond with the three distinct divisions of the year under a mediterranean climate: winter, spring and summer (Miller 1983). From the study by Bell and Stephens (1984) and others like

it (eg. Specht *et al.* 1981) examples are available of mediterranean Australian plant species growing in all months of the year. However, comparatively few studies exist for the period of late summer/autumn when soil moisture deficits are high (Specht & Rayson 1957a, Lamont 1981, Specht *et al.* 1981). One such study (Specht and Rayson 1957a) showed that for the large sclerophyllous species *Banksia ornate* in mediterranean southeastern Australia the main growth flush occurred during summer, with a pattern of continuous annual growth. Summer soil moisture under this community was concluded to be just sufficient to maintain the vegetation in a dormant state, let alone support vigorous growth (Specht 1957b). Since soil moisture was only measured to 2 m, within which most roots were found (Specht & Rayson 1957b), Bettenay's (1984) suggestion that summer growth is likely to be related to the amount of water stored in the underlying substrate can not be ruled out.

Although the definition of the Mediterranean-type climate can vary depending on the author (see Orshan 1983) it is accepted that growth is primarily limited by water (Miller 1983, Orshan 1983, Beard 1984, Lamont & Bergl 1991, Hobbs *et al.* 1995a, Larcher 2000). For the dominant sclerophyllous species, drought-tolerating adaptations permit shoot growth during the typical spring/summer-growing season. These include extensive medium to deep root systems, often involving a tap root and well developed laterals (Dodd *et al.* 1984, Hobbs *et al.* 1995a). A wide range of shoot and leaf forms have been adopted to cope with water stress, including structures promoting rainfall interception and channelling (Specht 1957a, Nulsen *et al.* 1986), and unusual displays of leaf dimorphism (Groom *et al.* 1994b). Similar physiological mechanisms abound. Decreased stomatal conductance (Crombie *et al.* 1988) or complete stomatal closure (Dunn 1975, Tenhunen *et al.* 1981) may regulate water loss, and osmotic adjustment provides a means of maintaining turgor under drought conditions (Bowman & Roberts 1985, Richards & Lamont 1996). If periods of prolonged drought occur, water stress exacerbated by excess heat loads will often force plants to cease growth and assume a state of dormancy that conserves water and enhances survival until relief by sufficient rainfall in late autumn.

Beard (1983) recognised three mediterranean regimes within the South West Botanical Province of Western Australia, representing a progressive drying trend in a north-easterly direction. However, unlike some mediterranean regions of the world where

water is limiting nearly all year round, even in the Extra-dry Mediterranean regime sufficient rainfall to relieve drought stress (300–500 mm annual rainfall, Beard 1983) is usually experienced during a period of 4 or 5 months. When water is not restrictive, growth can still only proceed as fast as permitted by the next most limiting factor. Of the various climatic components, temperature is regularly cited as an important variable limiting growth during either thermal extremes of the year, inhibiting growth in winter and contributing to water stress in summer (Groves 1965, Orshan 1983, Specht & Specht 1999). The role of temperature in inducing and releasing species from forced winter dormancy between these climatically-contrasting periods has also been identified (Perry 1971, Flint 1974, Kummerow *et al.* 1981). Growth requires higher temperatures than photosynthesis, which has a very low temperature threshold (when CO_2 uptake ceases) coincident with ice formation in the mesophyll (Larcher 2000). In southern Californian chaparral vegetation, growth of the dominant species is restricted when daily mean temperatures fall below 10 ˚C (Miller 1983), and similar low temperatures during the winter months inhibit growth of the dominant species in the maquis-garrigue vegetation in southern France (Specht 1969). Several studies of Australian mediterranean environments have indicated that growth of the dominant tree and shrub species is initiated when mean air temperature exceeds 16–18 ˚C (Specht & Rayson 1957a, Groves 1965, Specht 1973, Specht *et al.* 1981). This minimum threshold is less (13 ˚C and even 11 ˚C) for subdominant and understorey heath species (Specht *et al.* 1981), which generally have an earlier growing season.

Specht and colleagues have compiled evidence (Specht & Dettmann 1995, Specht & Specht 1995, Specht & Specht 1999) suggesting that the overstorey species in mediterranean Australia have undergone little change since the more humid and warmer climate during the early Tertiary. As a consequence these species have retained a subtropical (mesotherm) growth rhythm, exhibiting peak growth in late spring/summer. However, the growth response of the understorey stratum has evolved more recently becoming closely synchronised with the cool, humid winter-spring season of the mediterranean climate (mircotherm) (Specht & Dettmann 1995). Specht and Dettmann (1995) explain that the understorey species have compensated for a decrease in environmental temperature by the evolution of a more intricate leaf distribution structure. A reduction in leaf and internode size increases the stem-air boundary layer, impeding heat dissipation and effectively raising the temperature of the growing apices

by more than 5 ˚C. Physiologically, understorey species respond to the same temperature range as the overstorey. However, the increase in the boundary layer prematurely warms the understorey species resulting in their earlier response to rising spring temperatures.

This interpretation lacks an explanation of the environmental pressures responsible for the bias in the evolutionary reduction of leaf size towards understorey species. Dry environments throughout the world experiencing high radiation and temperature loads, such as Mediterranean-type ecosystems, have been repeatedly correlated with flora exhibiting small leaf dimensions (Parkhurst & Loucks 1972, Grime 1979, Fahn 1990). The processes of natural selection driving this reduction in leaf size has been attributed to greater efficiency in convective heat loss by smaller leaves, preventing the disruption of cell metabolism and general tissue damage due to excessive leaf temperatures (Crawley 1986). Transpirational water loss can also increase with increasing leaf size (Taylor 1975), as will usually be the case under a mediterranean climate (Miller 1983) with its high summer-autumn temperatures. The greater boundary layer of leaves with larger size dimensions contributes significantly to these relationships (Taylor 1975), which contradicts Specht's reasoning. However, this counter argument does not consider crown structure that is a crucial part of Specht's explanation.

Because of the marked resprouting response of grasstrees to fire most prior work has concentrated on this aspect, with little literature on seasonal growth phenology in the absence of fire. However, a few studies are available that offer clues to the likely growth phenology of *X. preissii*. Continuous annual leaf growth of the Western Australian grasstree *Kingia australis* (family Dasypogonaceae) has been documented (Lamont 1981). Annual fluctuations in stem diameter in *X. preissii* suggest seasonal changes in growth, with most leaf development occurring during winter-spring (Lamont & Downes 1979). But, this information is not specific enough and lacks the essential detail to thoroughly meet my aims. The main aim of my study was to determine the growth rhythm of *X. preissii* independent of any recent disturbance by fire, and to do this in two contrasting habitats so that the results may have wider application. For some time it has been widely known that grasstrees exhibit slow stem elongation (Herbert 1920, Lewis 1955, Gill & Ingwersen 1976, Lamont & Downes 1979, Ward *et al*. 2001). Therefore, the likely futility of completing a detailed assessment of *X. preissii* growth phenology from stem elongation determined my study's focus on leaf growth and production. To

complement this work, root growth patterns were also investigated, thus providing a complete picture of vegetative grasstree growth. Older grasstree roots existing horizontally in the upper soil layers typically consist of a loose black sheath, remnant of the originally fleshy cortex, encompassing the wiry stele, complete with intact and open conducting vessels. The term stele used here has been described in greater detail, as consisting of the actual stele surrounded by endodermis and then tightly bound by a strong sheath of lignified fibres (Pate and Canny 1999). Young roots with an intact fleshy cortex can elongate and are usually unbranched, and in older plants new rings of roots are initiated progressively higher on the stem (Staff & Waterhouse 1981). Previously, root growth rates and phenology in grasstrees has only been reported for the species *Kingia australis*, which, unlike *X. preissii,* initiates roots from the stem apex (Lamont 1981).

My aim was twofold: 1) to assist in verifying the grasstree ageing and fire history technique (Ward *et al.* 2001), and 2) to identify the annual pattern of grasstree growth, from which to later distinguish any effect of fire (Chapter 3). The ageing part of the technique described by Ward *et al.* (2001) assumes that the alternating cream and brown bands comprising the exposed ends of the leafbases represent seasonal changes in growth. This implies *X. preissii* experiences two distinctive alternating phases of growth during each year that control leafbase colour. Traditionally trees growing in extreme conditions have been used for conventional dendroecological studies, as they respond strongly to climatic variation (Fritts 1976), suggesting that the contrasting seasons of the mediterranean climate may help interpret the banding pattern.

For the second part of my aim it was considered important to understand the underlying phenophases that occur regularly with the changing seasons, which, would allow me to make interpretations regarding the effect of fire on grasstree growth (see Chapter 3). Only after the effects of everything except fire can be accounted for is it possible to reasonably assess fire effects. Flint (1974) provides a comprehensive review of the environmental factors that control phenological events including dormancy. His work emphasises the controlling effect of temperature on the process of growth initiation following winter, and photoperiod on the cessation of growth and induction of dormancy. Although *X. preissii* was considered unlikely to experience a true period of dormancy during summer drought or winter, a strong seasonal pattern of growth

(Lamont & Downes 1979) could be viewed as an analogous phenomenon. On this basis, strongly seasonal climatic variables such as temperature, rainfall and daylength were considered as possible controls of grasstree growth. The extension of this study to include an interpretation of the climatic variables that govern vegetative growth was aimed at enhancing our level of understanding of grasstree phenology. The effect of habitat type on these interpretations is also discussed.

Encompassing the two levels of my main aim, the following hypotheses were investigated during this study:

1) The distinct summer and winter seasons of the mediterranean climate of southwestern Australia dictate the strong bimodal pattern of annual growth in *X. preissii*.
2) Seasonal changes in temperature and rainfall have the greatest control of grasstree growth.
3) The number of leaves produced in one year equals the number of leafbases contained in two adjacent cream and brown bands.

2.2 Materials and methods

2.2.1 Study sites

Unburnt sites located in jarrah (*Eucalyptus marginata*) forest on the Darling Range (MC; Fig. 1.2a) and banksia woodland on the Swan Coastal Plain (YC; Fig. 1.2b) were studied. These two sites (Fig. 1.3) are described in Table 1.1. Temperature and rainfall data were obtained from the Perth Bureau of Meteorology for Bickley, the closest meteorological station to the jarrah forest sites. For the banksia woodland sites, temperature data were obtained for Lancelin and rainfall data from Yanchep National Park (Department of Conservation and Land Management, CALM) records. These data are given in Figure 2.1. Additionally, daylength (the number of hours per day for potential photosynthesis) and proportion cloud cover were obtained for the Bickley and Lancelin weather stations and incident radiation was provided for the specific site

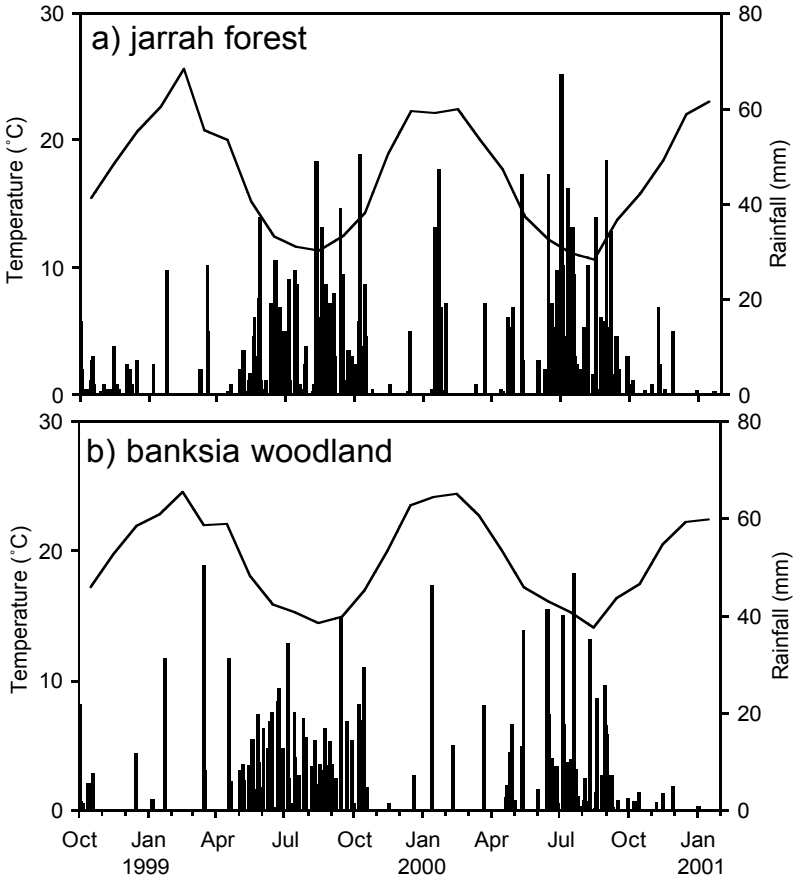

Fig. 2.1 Mean daily temperature and daily rainfall for the two habitats studied. Data were obtained for the closest recording sites to the study area from the Bureau of Meteorology and the Department of Conservation and Land Management (1998–2001. See text for details).

coordinates to assist in the interpretation of leaf growth. Global daily exposure to solar radiation is an estimate based on satellite data, and is derived from physical modelling of radiation transfer within the atmosphere (considering parameters such as cloud cover), and offers 5–8% accuracy (Weymouth & Le Marshall 1999).

2.2.2 Direct measurement of leaf production and elongation

Monitoring of leaf production and elongation for *Xanthorrhoea preissii* commenced on 30 October 1998 in the jarrah forest and 5 November 1998 in the banksia woodland. At each site, six specimens of *X. preissii* within the height range 0.8 to 1.25 m (a convenient working height measured from the ground to the terminal apex) were randomly selected from 100 grasstrees previously marked.

The leaves of *X. preissii* can be divided into four categories relative to their maturity: 1) creamy white, recently emerged young leaves, 2) light green intermediate leaves not of full length, 3) full length, dark green matured leaves, and 4) dry, brown dead leaves. For each plant the three live leaf categories were tagged with a different coloured electrical cable marker (Critchley Pty Ltd., Castle Hill, Australia) for identification (young = 1 of 11 colours indicating season of initiation, intermediate = orange, and mature = white; Fig. 2.2). All juvenile leaves were tagged, counted and recorded as they became visible. A representative sample of 60 intermediate leaves was tagged in two strips of 30 leaves radiating out from the boundary of the previous leaf category to the boundary of the next category. The mature leaves had two similar strips of 25 leaves tagged. In both cases the two strips faced north and south and represented the range of leaf ages present in the category.

Fig. 2.2 A grasstree crown spread open to reveal the coloured markers (2 mm long) used to tag newly initiated leaves for monitoring leaf production. Note the innermost red tags were applied at the time of the photograph and represent leaves initiated during winter 1999, while the yellow tags in the upper portion were used prior to these to mark those leaves initiated in the previous season, autumn 1999.

All sites were visited every 13–58 days, determined by the rate of leaf production. On each visit all newly emerged young leaves at the apex of each grasstree were tagged with the appropriate coloured cable marker and the total number recorded. The rate of leaf progression from one category to the next was also recorded as a measure of rate of leaf elongation, since leaf length is a primary characteristic used to distinguish the three live leaf categories. Progression of young leaves into the intermediate category was calculated by subtracting the number of young leaves remaining in that class from the total number of young leaves tagged since time zero, taking into account the number of young leaves progressing from the period before. Leaf maturation was determined on a relative basis by counting the proportion of the original 60 orange-tagged intermediate leaves progressing to the mature category, and adding a white tag to denote its progress.

For each leaf tagged white, one of the smallest new intermediate leaves was tagged orange to retain the original number of 60. Age at maturity was calculated from the time of initiation (identified by the colour of the young leaf marker present) of each intermediate leaf that matured. Leaf mortality was measured in a similar way, by counting the number of white-tagged mature leaves that died during each inter-visit

period. As for the maturing leaves, the season of initiation was recorded for the dead leaves (for the calculation of age at death) and an extra white tag applied to indicate that it had been counted. In addition, total leaf death and total leaf production was compared at each site (MC and YC). An additional four grasstrees at each site had their smallest young leaves tagged so that all new leaves produced after one year (July 1999 to July 2000) could be counted. For the same plants a small amount of the youngest thatch was removed in July 1999 so that after one-year, in July 2000, all leaves that had died could be counted.

Rates of leaf production and leaf progression (≅ elongation) were calculated. Three complementary approaches incorporating statistical and descriptive methods were used to explain the relationship between seasonal patterns of leaf production and climatic variables: 1) simple correlation and detailed description, 2) modelling and 3) a novel approach introduced by Specht (1995) to define plant growth rhythms within Australia as a function of temperature. All assumptions of the statistical tests used below were met, including testing for autocorrelation using the Durbin-Watson test (Fry 1993), and examination of residuals to ensure that the assumptions regarding homoscedasticity, normality, linearity and outliers (univariate and multivariate) were adhered to. Statistical analyses were performed using both Statistical Analysis System (SAS/STAT software, SAS Institute, North Carolina, USA) and SPSS 10.0 (SPSS Inc., Chicago, U.S.A.) software programs. Leaf production was systematically correlated with a number of climatic variables (temperature, rainfall, incident radiation, daylength and proportion cloud cover), revealing to which variables leaf production was most sensitive.

From these findings the two best-fit, biologically-meaningful, leaf production models were determined for each habitat using multiple linear regression techniques. The first predicted a full year of leaf production based on a single regression equation, while the second incorporated two separate season-based regression equations to explain data of the full year. The decision to build the second model was based on an attempt to improve the coefficient of determination (r^2) from the first model, by separately modelling the distinct characteristics of the summer fast-growing season and the winter slow-growing season, and combining the results. Where the interaction term "mean daily temperature × total rainfall" was included in the regression model, the response surface of temperature plotted against predicted leaf production for different rainfall amounts

was used to interpret this effect. The relationship was tested for linearity using the Box-Tidwell transformation (Hosmer & Lemeshow 1989) on each variable, to ensure it was suitable to include in a linear regression. For annual leaf production predicted from the two seasonal regression equations an overall r^2 was calculated from the predicted and actual leaf production data. Semi-partial correlations were calculated for the two single regression models to determine the individual contribution made by each variable to explain leaf production. The model's predictive power was estimated using the r^2 PRESS (predicted error sum of squares) statistic. This statistic is a more accurate estimate of the predictive power of a model than r^2 (which is specific to the data from which it was created). As a final test of the predictive power of the jarrah forest regression models a comparison was made between actual leaf production data collected over 273 days (not used in creating either model), and leaf production values predicted from environmental data using the two regressions.

2.2.3 Indirect measurement of leaf production

Another six randomly-selected grasstrees from the unburnt jarrah forest and banksia woodland sites were used for the indirect assessment of annual leaf production. This method involved the removal of all leaves, both dead and alive, and the identification of the annual growth bands on the stem (Ward *et al*. 2001). Leaves were removed and ground back to their bases using a 100 mm angle grinder powered from a four-stroke petrol generator. Similarly, the charring on the leafbases left by past fires was removed to reveal the colour of the leaf bases underneath, using the method described by Ward et al. (2001). A count was then made (for the entire circumference) of the number of leafbases contained in each of the two most recent, clearly identifiable, brown plus cream growth bands. The mean of each pair of bands was used as an indirect measure of annual leaf production for each grasstree selected. This count was compared with those results from the direct approach and a two-way ANOVA was conducted to determine the level of significance.

2.2.4 Root growth

To complement the above ground growth measurements, an effort was made to monitor seasonal root growth activity. Due to the difficulty in penetrating the lateritic soils on the Darling Range, the investigation of grasstree roots was conducted exclusively on the

Swan Coastal Plain, where the sandy substrate was conducive to root excavation. From June 1999 to January 2000 considerable problems using a method of repeated excavation of the same roots over time were met, so a new strategy for measuring root growth was implemented during 2000. Because the root system of *X. preissii* is quite extensive (Crombie *et al.* 1988), the second method used sampling of random grasstrees to collect data on the production of new roots, providing a general indicator of root growth activity. Grasstree roots were sampled at the unburnt banksia woodland site (YC), and on each occasion no fewer than five grasstrees were excavated, in an attempt to sample a reasonable number of plants with new roots. Soil surrounding the stem of *X. preissii* was removed to a depth of 20–30 cm, uncovering the shallowest roots. New roots were consistently produced adjacent to the shallowest existing roots, and were distinguished from these older roots by scratching the outer surface of all roots encountered with a scalpel to examine the cortex. The cortex of older roots (dead, brown and corky, or may have totally disintegrated leaving only the wiry stele) was quite different from the cortex of young new roots (bright green, soft and fully hydrated). Using this method of classification a count of the total number of new roots was made, and at least half of these roots were completely excavated and excised close to the stem, storing them in a cool bag during transportation. The length of each root was measured and the condition of the growing tip was recorded for each root as one of three categories of health: growing, dormant or rotten (Fig. 2.3). Roots were then washed in water to remove sand particles, bagged and dried at 70 ˚C for 60 hours or constant weight to determine dry mass. This process was repeated once in each season of the year spanning 2000 to 2001 (August, November, February and May). Small sample size did not permit the use of chi-squared analysis to detect the effect of season on the occurrence of new roots. For this reason the binomial test was used to determine if the probability of *X. preissii* having new roots was enhanced or decreased in any particular season from the overall expectation of 0.5. In addition, the percentage of grasstrees with new roots and the proportion of new roots in each condition category were determined. For the grasstrees that had new roots, mean estimated total root biomass (mean new root dry mass × number of new roots), mean number of roots per grasstree and mean root size (g/cm) were calculated, and significance of differences between seasons was determined using one-way ANOVA. The non-parametric Kruskal-Wallis test was used to analyse the new root biomass data, as transformation did not allow the data to meet the assumptions of ANOVA.

Fig. 2.3 Condition of new root tips (from left to right): healthy, actively growing; slow growing; dormant; and rotten with scale insects (seen as pink/white oval structures) present. Fine graduations on the scale are millimetres.

From observations made at a recently excavated new housing estate (Ocean Keys), within the northern suburbs of the Perth metropolitan area, it was possible to estimate rooting depth of *X. preissii* on the Swan Coastal Plain. Following excavations at the estate a group of grasstrees was left on the edge of the cleared land, and immediately within the cleared area it was possible to locate grasstree roots (Fig. 2.4). From the height difference between the original ground level where the living grasstrees existed and the modified ground level where the roots were found it was possible to estimate the depth grasstree roots could penetrate. To aid this estimation topographic maps and aerial photographs of the area prior to ground works were consulted, courtesy of the developer's landscape architects (Hames Sharley, Subiaco).

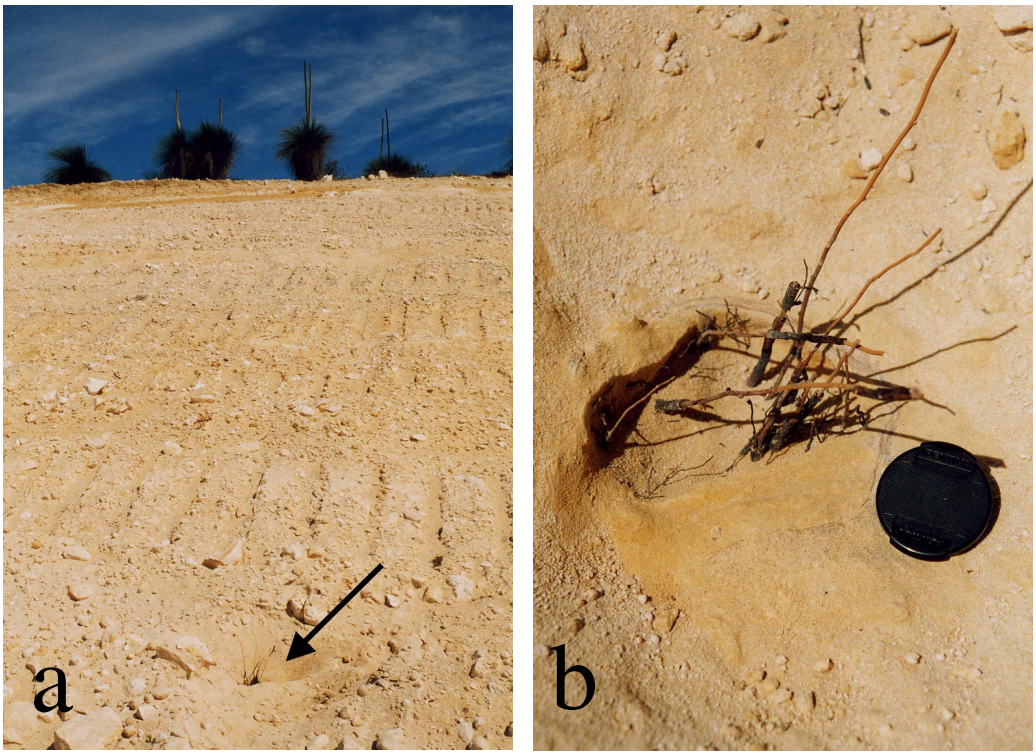

Fig. 2.4 a) *X. preissii* growing adjacent to earth excavations at a new housing development site, with associated roots indicated in the foreground, 6.4 m vertically below the surface. b) Close-up view reveals growth of these old decorticated roots down through a hole in the limestone substrate (lens cap is 5 cm in diameter).

2.2.5 Grasstree water relations

2.2.5.1 Seasonal water potentials

Water potential (Ψ) was initially measured on each category of leaf (young, intermediate and mature) on grasstrees on the Darling Range and Swan Coastal Plain, to determine if leaf age significantly correlated with water potential. This preliminary work indicated a difference between young leaf Ψ and intermediate and mature leaf Ψ, which was attributed to the vertical aspect, self-shading and immaturity of the young leaves. From this work, the intermediate leaf category was chosen for continued Ψ measurements, as these leaves were neither young nor old, and constituted the majority of leaves on the plant.

Xylem water potential (Ψ_X) was measured predawn (Ψ_{PDX}; just prior to sunrise) and midday (Ψ_{MDX}; following 12 noon) for grasstrees at the same two sites used for leaf growth monitoring. Measurements were made from 20 November 1998 to 5 June 2001 in the jarrah forest and from 24 November 1998 to 30 March 2000 in the banksia

woodland. The last 15 cm of one intermediate leaf (length restricted by the pressure chamber dimensions) was collected from six grasstrees selected randomly from the available plants, for measurement of Ψ_X using a pressure chamber (PMS Instrument Company, Oregon, USA) as described by Scholander *et al*. (1965).

In order to assess the extent of summer water stress on *X. preissii*, water potential at turgor loss point (Ψ_{TLP}) was determined for a sample of grasstrees at each site using the method described by Tyree and Hammel (1972). One leaf tip (15 cm) was collected from six randomly selected grasstrees predawn during mid-autumn (3 April 2000 for the jarrah forest, and 30 March for the banksia woodland). The leaf was excised underwater and then transferred to a sealed container, allowing the leaf to stand vertically in florist's foam immersed in water in a darkened environment. The leaves were retained in this state for 3 hours before an initial weighing (turgid weight) then subjected to gradual drying under laboratory conditions. During the drying process, leaves were weighed before and after the measurement of balancing pressure, using a pressure chamber. This series of operations was repeated with short periods in between (< 3 minutes). The data collected were used to calculate the mean water potential at turgor loss point, by plotting relative water content (leaf weight / turgid weight) against the inverse of the balancing pressure.

2.2.5.2 Response to simulated summer rain

Leaf production and Ψ_X was measured for six unburnt randomly-selected grasstrees from the banksia woodland (YC) before and after a single watering treatment, equivalent to 59.2 mm of rain within a 2.5 m radius of the stem. This amount of water represents about 3/4 of the highest single rainfall event recorded for Lancelin (78.6 mm) during April, and almost twice as much as the mean size of a single April rainfall event (32.1 mm; Bureau of Meteorology). On 20 April 2001 ground water collected and stored (by CALM, for fire fighting) at a site within 5 km of YC was transported to YC using a CALM water truck, with a 2700 L capacity. Three truckloads of water were sprayed over the plants and surrounding ground using a coarse spray nozzle, at a rate of 0.9 L/s (Fig. 2.5). Leaf production was monitored from 18 January 2001 (as described in Section 2.2.2), until 26 days after the watering treatment. Prior to watering, Ψ_X was measured for the grasstrees on 3 occasions (29 January, 19 February and 20 April), and once the morning after the watering treatment. Soil water potential (Ψ_S) at depths of 13,

23, 33 and 43 cm was measured concurrent to Ψ_X at four locations within the study site. At each location a straight-sided 30 cm diameter hole was dug and a psychrometer sensor inserted into the side of the hole at each depth, before filling the hole (maintaining original stratification of the soil) followed by gentle tamping. Psychrometer locations were also watered with the equivalent of 59.2 mm of water, and Ψ_S was measured concurrent to Ψ_{PDX} using a HR-33T dew-point microvoltmeter (Wescor Inc., Utah, USA). Rainfall at the site was measured using a single rain gauge.

Fig. 2.5 Water being applied to a grasstree and the surrounding ground within a 2.5 m radius (marked by red flagging tap), as a simulation of rainfall. The time taken to fill the 46 L bin in the foreground was used to estimate the rate of water flow, so that the correct volume of water could be applied to the area.

2.2.6 Statistical analyses

Special data analysis techniques are outlined in the relevant methods sections. However, where routine statistical tests were performed (ie. *t*-test and ANOVA) and were not described, the analyses were conducted using SPSS 10.0 software program (SPSS Inc., Chicago, USA). Analysis type used is indicated with the reported results, and $\alpha < 0.05$ was used to determine significance. Normality was tested by examining stem-and-leaf plots of the residuals, as well as calculating the Shapiro-Wilks statistic (Coakes & Steed 1999). Levene's test was employed to test for homogeneity of variances for all data. Based on the outcome of this data screening, data were either square root or log transformed as required and then re-analysed. All transformed data are presented as untransformed means, and all error terms provided are standard error ($s/\sqrt{n}$).

2.3 Results

2.3.1 Direct measurement of leaf production, elongation and death

2.3.1.1 Seasonal patterns of leaf production

The Durban-Watson test indicated that both leaf production data sets were not autocorrelated. This was sufficient evidence to support the assumption that the data were independent, despite their repeated-measures nature (Matt Williams, biometrician, CALM, pers. comm.). In the following, it was considered reasonable to perform regression analysis between climatic variables and leaf production as a method of determining their relationship.

X. preissii shows clear oscillations in leaf production for both habitats (Fig. 2.6a,b). Leaf production was at a maximum from late spring (November) to autumn (April/May), persisting a little longer in the banksia woodland. During this period highest leaf production (2.5 leaves/d in jarrah forest and 3.2 leaves/d in banksia woodland) occurred at a mean daily temperature of 25.5 ˚C in jarrah forest and 23.3 ˚C in banksia woodland. Through the winter months and early spring leaf production was at a minimum, maintained close to one leaf per day. At no point during the year did leaf production fall to zero; the lowest growth recorded was 0.17 ± 0.07 leaves/d in the jarrah forest and 0.21 ± 0.08 leaves/d in the banksia woodland, both during March 1999. Over time it became clear that the jarrah forest grasstree seasonal pattern of leaf production was more pronounced than that of the banksia woodland plants (Fig. 2.7). Because of this pattern of leaf production, daylength, the most seasonal of the available variables, was investigated as a possible control of leaf production. Daylength significantly contributed to explaining leaf production, and although weak, on a relative basis, the relationship between leaf production and daylength for the jarrah forest grasstrees ($P < 0.0001$; ANOVA. Fig. 2.8a) was considerably stronger than that of the banksia woodland grasstrees ($P = 0.0312$; ANOVA. Fig. 2.8b).

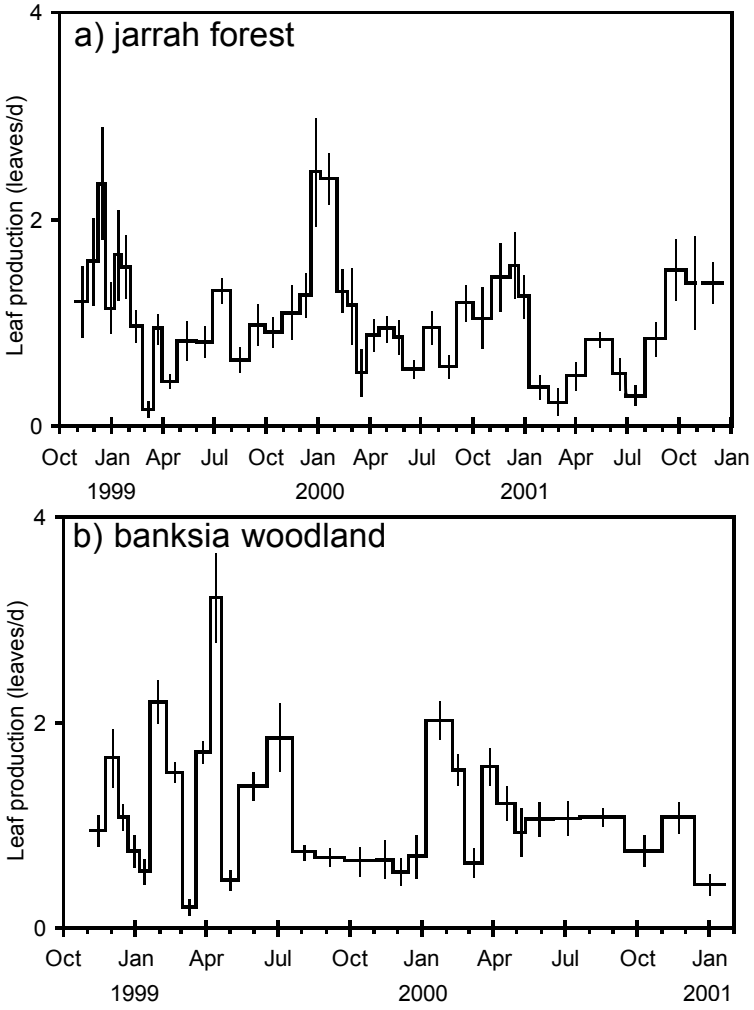

Fig. 2.6 Leaf production rate of *X. preissii* (1998–2001) in (a) jarrah forest and (b) banksia woodland. Data are the mean of six plants for each measurement period (horizontal line) ± SE.

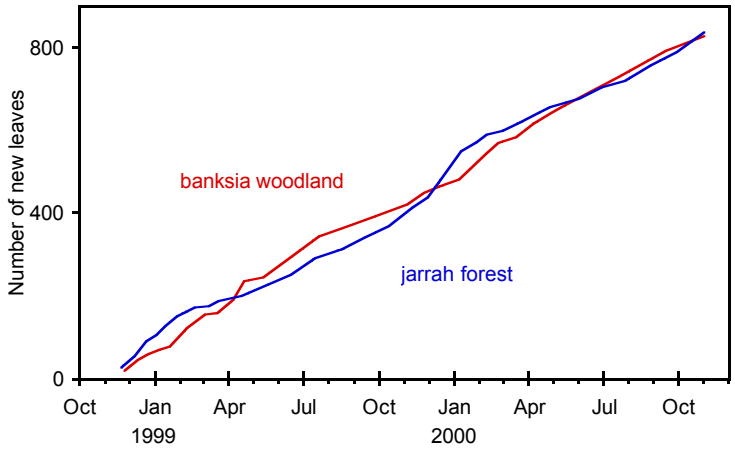

Fig. 2.7 Formation of new leaves by *X. preissii* over time in jarrah forest and banksia woodland. Data are the mean (n = 6) number of accumulated leaves.

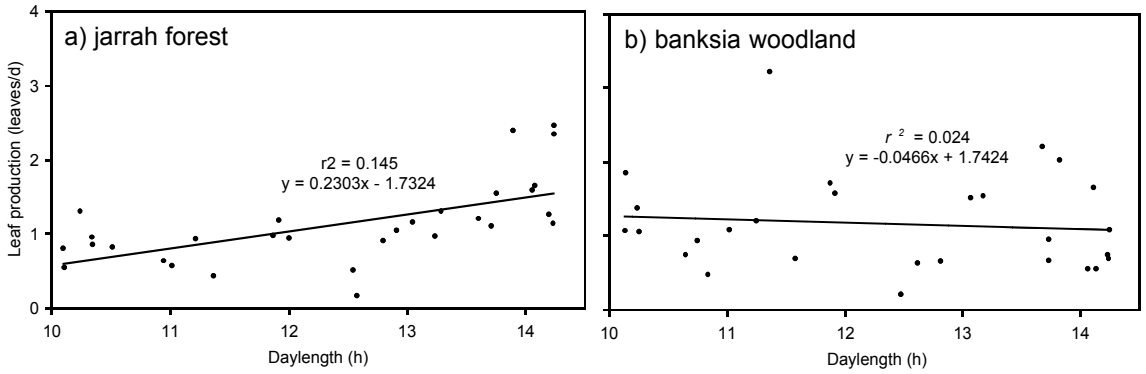

Fig. 2.8 Relationship between daylength and leaf production by *X. preissii* in (a) jarrah forest and (b) banksia woodland. Leaf production data are the mean of six plants for each measurement period, and daylength is given as the mean for the measurement period. For both relationships the line of best fit is plotted and the corresponding regression equation and r^2 value (derived from all data) are given.

The summer fast-growth phase was characterised by a fluctuating pattern of leaf production, particularly in the banksia woodland. This erratic growth was coincident with sporadic single summer rainfall events (Fig. 2.1) derived typically from a combination of moist air and instability in the mid-level troughs that can form along the west coast (Glen Cook, meteorologist, Perth Bureau of Meteorology, pers. comm.). From observing grasstree leaf production in the banksia woodland, these plants appear to be particularly sensitive to single rainfall events of about 18 mm or more (Fig. 2.1 and 2.6b, also see Section 2.3.3.2 on this topic), but this relationship is not clear in Fig. 2.9a. This phenomenon is obvious during the driest period, from about mid-January to May, but a similar response also occurred once after the first winter rains in 1999 when mean daily temperature was below 16 ˚C (seen as a 'slow growth' point at 34.2 mm in Fig. 2.9a). The positive relationship between leaf production and rainfall during the fast-growing season ($P < 0.0001$; ANOVA) did not exist during winter, where the slope of the regression equation was not significantly different from zero ($P = 0.2531$; ANOVA). This seasonal difference is clearly shown in Fig. 2.9a. As a consequence of the seemingly shorter growing season for the jarrah forest grasstrees the number of incidences where an increase in leaf production was observed to follow summer rainfall was low (only 3 definite occurrences over three years). On these few occasions, the stimulation of grasstree leaf production by summer rainfall was evident (Fig. 2.6a), although overall, a consistent relationship was not apparent (Fig. 2.9b. $P = 0.3202$; ANOVA). Similarly, the correlation between the size of winter rainfall events and leaf production was weak (Fig. 2.9b).

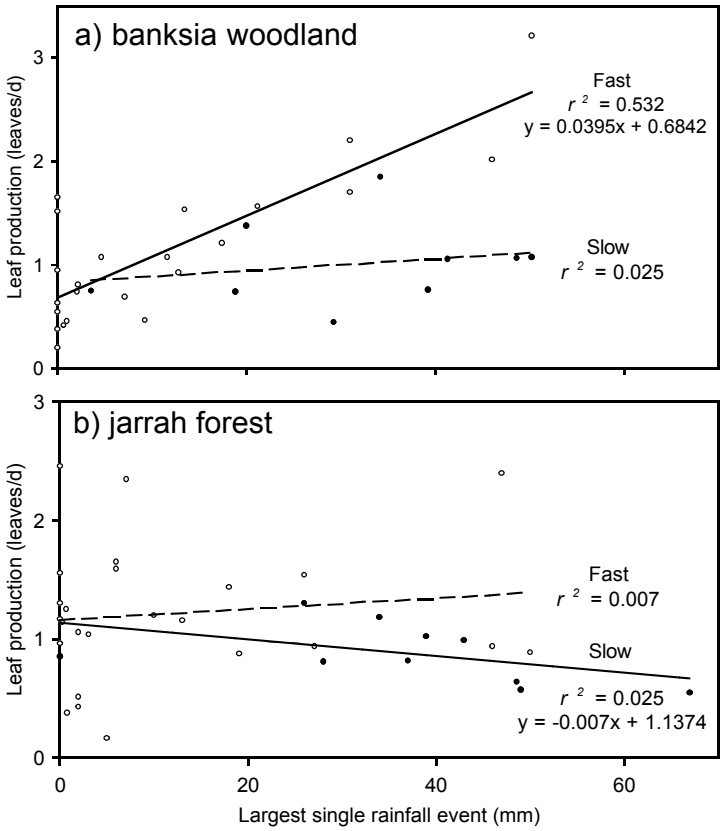

Fig. 2.9 Relationship between the single largest rainfall event during each measurement period and leaf production of *X. preissii* in (a) banksia woodland and (b) jarrah forest. Data for each habitat have been separated into slow-growth (mid-May to mid-November; ●) and fast-growth (mid-November to mid-May; ○) periods. Leaf production data are the mean of six plants for each measurement period, and rainfall is the greatest rainfall amount to fall within each measurement period. For each relationship the line of best fit is plotted (broken if not significant) and the corresponding regression equation and r^2 value (derived from all data) are given.

In both habitats the commencement of the summer growing season was prominently marked by a sudden rise in leaf production (Fig. 2.6), but accurately predicting this point was difficult because of the variability of its timing. Generally, the start of this growth phase was correlated, in both habitats, with a mean daily temperature exceeding 20 °C (eg. 1998/99 growing season), but this relationship was unreliable when there was poor rainfall from mid-October in the lead-up to the season (eg. 1999/00 growing season). This temperature threshold was consistently reached during mid- to late-November, which was characterised as having the greatest increase in ambient temperature during the year. After the fast-growing season, with the transition from autumn into winter, the boundary between the faster leaf production period and the start of the slower winter

growth was far more difficult to distinguish. Generally, by the end of May, leaf production had attained the slow growth associated with winter. In contrast to November, it is in May that the greatest decrease in ambient temperature was recorded for the year.

The first attempt to model annual leaf production used a single regression equation. To attain the highest r^2 values a different set of climatic variables was used to model leaf production in each habitat. In the jarrah forest, leaf production was positively correlated with total rainfall received in each monitoring period and the mean daylength of the period (see regression equation below). Independent of daylength, temperature was correlated with leaf production. However, it was not used in the model as significant overlap existed in the portion of leaf production variance explained by both variables. Banksia woodland grasstree leaf production was negatively correlated with total rainfall, but positively correlated with mean daily temperature and the interaction of temperature and rainfall. Biologically, this interaction describes a positive effect of temperature on growth that increases when water is not limiting (Fig. 2.10). Linear regression equations for both habitats are given below:

Leaf production (Jarrah forest) = (0.002 × Rain) + (0.271 × Daylength) – 2.389

Leaf production (banksia woodland) = (0.051 × Temperature) – (0.042 × Rain) + (0.003 × Temperature × Rain) – 0.347

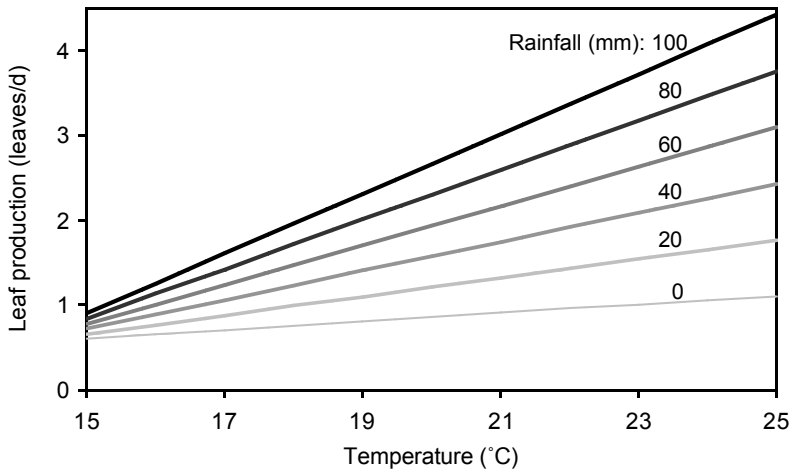

Fig. 2.10 The surface responses for the first leaf production model of banksia woodland grasstrees, showing the relationship between rainfall and temperature throughout the year. Leaf production data for this figure were calculated from the linear regression equation given in the text.

As these equations yielded the highest r^2 values it is not surprising that each variable, including the interaction term, significantly contributed to the prediction of leaf production (*t*-test, Table 2.1). In the jarrah forest, daylength was most closely correlated with leaf production, accounting for over 85% of the variation in leaf production predicted by this model, according to the squared semi-partial correlations (Table 2.1). For banksia woodland grasstrees, the combination of total rainfall and the interaction of rainfall and temperature explained the majority of variation in leaf production. r^2 values indicate that leaf production by the banksia woodland grasstrees was more precisely described by the climatic variables considered than leaf production by the jarrah forest grasstrees (Table 2.1). However, the proportion of variation in leaf production explained by these two models is quite low for both habitats. The difference in r^2 values for the two habitats is apparent when predicted leaf production was plotted with actual leaf production (Fig. 2.11a,b). The predicted values adequately fitted the actual leaf production profile for the banksia woodland grasstrees, whereas the fit was poor for the jarrah forest grasstrees, although correctly predicting a general seasonal trend. Consistent with this is the suggested predictive power of the respective models indicated by their r^2 PRESS statistics (Table 2.1). The jarrah forest regression was capable of predicting leaf production data (separate to those used to create the model) with a level of accuracy that highlighted the main seasonal trends (Fig. 2.11a).

Table 2.1 Results of multiple linear regression analysis, indicating *P*-values for the effect of daylength, rainfall, mean daily temperature, and the interaction of the last two variables on leaf production from November 1998 to February 2001, in two habitats. The squared semi-partial correlations are indicated in parentheses beside the *P*-values. The coefficient of determination (r^2) is given for each model created from these climatic variables. An estimate of the predictive power of each regression model is given (r^2 PRESS statistic).

Analysis	Jarrah forest	Banksia woodland
t-test		
Daylength	0.0001 (0.144)	
Rainfall	0.0267 (0.020)	0.0001 (0.134)
Temperature	–	0.0239 (0.016)
Temperature × rainfall	–	0.0001 (0.196)
r^2	0.166	0.402
r^2 PRESS statistic	0.144	0.372

Although total rainfall was correlated negatively with leaf production in the banksia woodland model, the net effect of rainfall (including the interaction) on leaf production appeared to be positive, evidenced by the adhesion of predicted values to the peaks and troughs associated with sporadic summer rain (Fig. 2.11b). Grasstrees in both habitats were characterised by at least two substantial deviations between the predicted and actual leaf production data. In most cases, the mismatch represents an underprediction by the regression models. The largest exception was the predicted value during spring 1999 for the banksia woodland grasstrees.

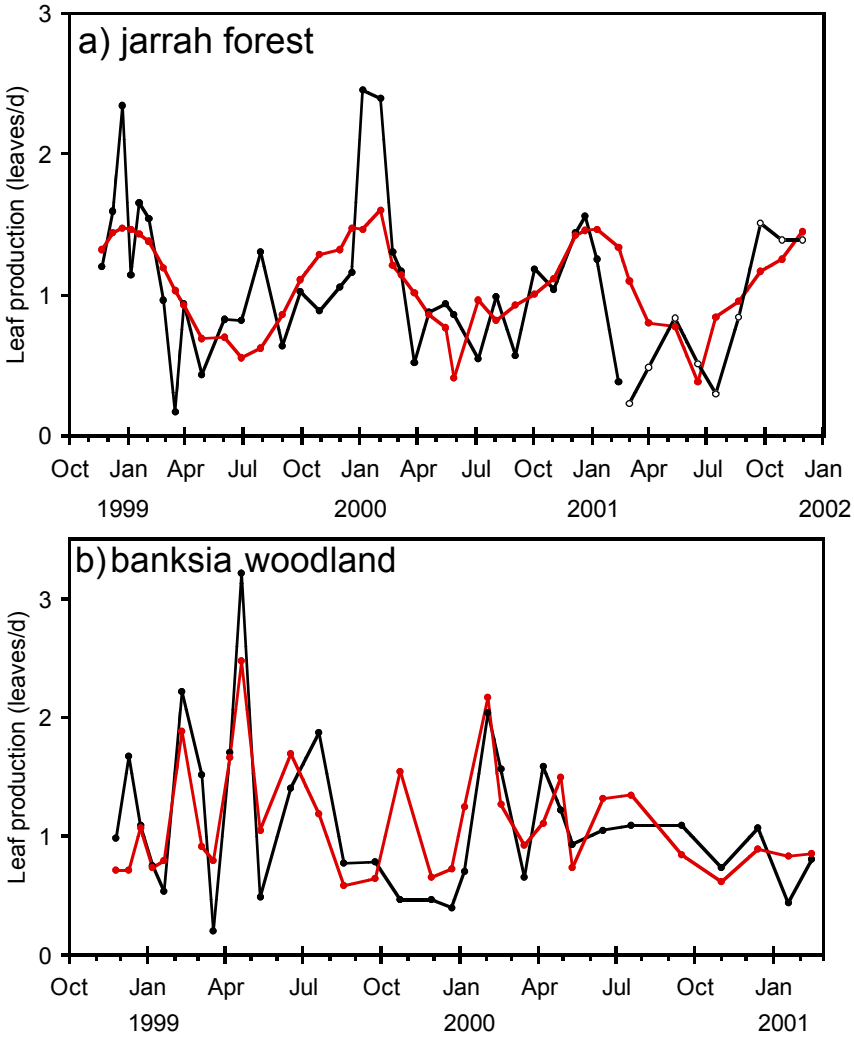

Fig. 2.11 Actual (●) and predicted (●) leaf production based on the first regression model, for *X. preissii* in (a) jarrah forest and (b) banksia woodland, over time (1998–2001). Actual data are mean leaf production of six plants over each measurement period, and the predicted leaf production values were calculated from the mean of each climatic variable over the same period. For the jarrah forest grasstrees, a second series of "actual" data are plotted (○); those that were not used in creating the regression model.

In trying to improve the two models described above, the year was divided into a summer growing-season and a winter non-, or more accurately, slow-growing-season, that were modelled separately. The choice of boundaries for the summer growing-season was based on the seemingly important minimum threshold temperatures discussed above. Mid-November and mid-May coincided with the chosen start and finish of the fast-growing-season respectively. Of the available variables, mean daylength and maximum single rainfall event for each monitoring period were most closely correlated with jarrah forest grasstree leaf production during the summer growing season ($P < 0.0005$ for both, t-test. $r^2 = 0.235$). Summer leaf production in the banksia woodland was most closely correlated with a solitary variable, maximum single rainfall event for each monitoring period ($P < 0.0005$, t-test. $r^2 = 0.530$).

Leaf production during the slow-growing-season for both habitats was poorly correlated with all variables, so the appropriateness of using a constant was investigated. The rate of jarrah forest grasstree leaf production was not significantly different between the winters of 1999 and 2000, but the rate of leaf production varied significantly between the six monitoring periods of each winter (Table 2.2). Examination of pairwise comparisons indicated that the two most contrasting periods of leaf production rate for the jarrah forest grasstrees were, at the very best, only marginally significantly different ($P = 0.052$, pairwise multiple comparison with Bonferroni correction).

Table 2.2 *P*-values from two-way repeated-measures ANOVA of winter (slow-growing) leaf production rates in two vegetation types. Leaf production rates recorded during 4 or 6 monitoring periods (six monitoring periods during each winter for jarrah forest, and four monitoring periods during each winter for banksia woodland) during two consecutive years (1999 and 2000) were compared.

	Jarrah forest	Banksia woodland
Monitoring period	0.001	0.001
Year	0.308	0.162
Monitoring period × year	0.439★	0.022

★ *P*-value adjusted to correct for violating the assumption of sphericity (Coakes & Steed 1999).

Similarly, for the banksia woodland no difference in leaf production rate existed between years, but a difference was apparent between the rates recorded during the four monitoring periods of each winter (Table 2.2). This difference was attributed to a significant difference between leaf production during July and October ($P = 0.002$, pairwise multiple comparison with Bonferroni correction). However, as the significant

interaction effect suggests, this was due mainly to an unusually high July 1999 leaf production rate (Fig. 2.6b). Prompted by these analyses, mean leaf production rate during the slow-growing season (inclusive of all years) was accepted as the predicted leaf production (constant) for that season, for both habitats. The r^2 between the new predicted leaf production data (derived from the two combined seasonal models) and the actual data was 0.261 for jarrah forest grasstrees, and 0.459 for banksia woodland grasstrees. These coefficients represented a clear improvement on the first modelling approach, particularly for the jarrah forest (Fig. 2.12a,b). Despite the improved r^2 for the second jarrah forest regression, it did not seem to predict the nine months of leaf production data (independent of those used to create the model) as well as the first model (Fig. 2.12a).

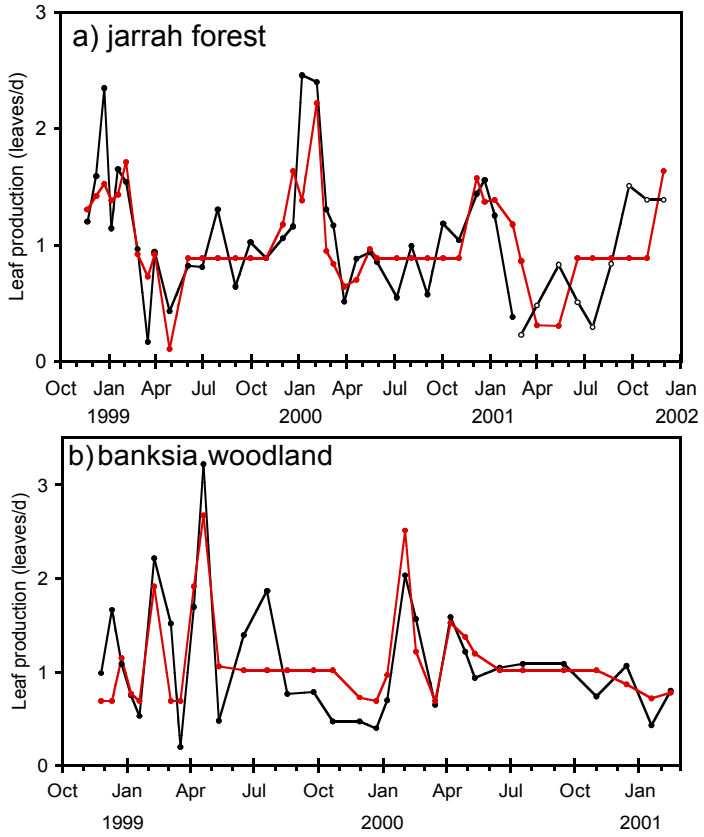

Fig. 2.12 Actual (●) and predicted (●) leaf production based on the second regression model, for *X. preissii* from the (a) jarrah forest and (b) banksia woodland, over time (1998–2001). Predicted values for summer and winter season are calculated from separate methods (see text). Actual data are mean leaf production of six plants over each measurement period, and the predicted leaf production values were calculated from the mean of each climatic variable over the same period (summer), or designated as a constant (winter). For the jarrah forest grasstrees, a second series of "actual" data are plotted (○); those that were not used to create the regression model.

Figures 2.13a–d (after Specht & Dettmann 1995, p. 208, Specht & Specht 1999, p. 185) define the relationship between mean daily temperature and the seasonal growth rhythm of *X. preissii*, distinguishing water-not-limited (Fig. 2.13a,b) and water-limited conditions for the leaf production data collected from November 1998 to February 2001 (Fig. 2.13c,d). The identification of these two conditions was based on whether the amount of rainfall received during each leaf production monitoring period was greater than or less than 18 mm, reflecting water-not-limited and water-limited respectively. Where water is not limiting, grasstree growth is positively correlated with temperatures of ≥ 17.5 ˚C in the jarrah forest and ≥ 16.5 ˚C in the banksia woodland. Below these minimum threshold temperatures leaf production was relatively constant; despite a 7 ˚C range of temperature change in the jarrah forest. Mean daily temperature remained above the minimum threshold for 5–6 months of the year in the jarrah forest (November to April), and for 7–8 months in the banksia woodland (November to June). The upper threshold temperature for grasstrees in the jarrah forest could not be determined, as mean daily temperatures > 21 ˚C were not observed concurrent with periods not limited by water. Even in the banksia woodland where the mean daily temperature reached > 24 ˚C an upper threshold was not apparent. In the absence of sufficient summer rain, leaf production was limited and fell below the one leaf per day baseline rate observed during the cooler months of the year (Fig. 2.13c,d).

The arrows in Figures 2.13a–d depict the natural hysteresis temperature-leaf production response curves for *X. preissii* during the year. Considering Figures 2.13a,b, where water is not limiting, the directions of these arrows for the jarrah forest and *banksia* woodland data are opposing. Jarrah forest grasstrees are characterised by a clockwise pattern, where leaf production during spring is marginally higher than leaf production during autumn over the same temperature range. Grasstrees in the *banksia* woodland exhibit an anti-clockwise pattern. In this habitat leaf production is higher during the months of autumn over the same temperature range during spring. Total incident solar radiation is higher during spring than in autumn (Fig. 2.14a,b), and so is positively correlated with grasstree leaf production in the jarrah forest, but is negatively correlated with leaf production in the *banksia* woodland.

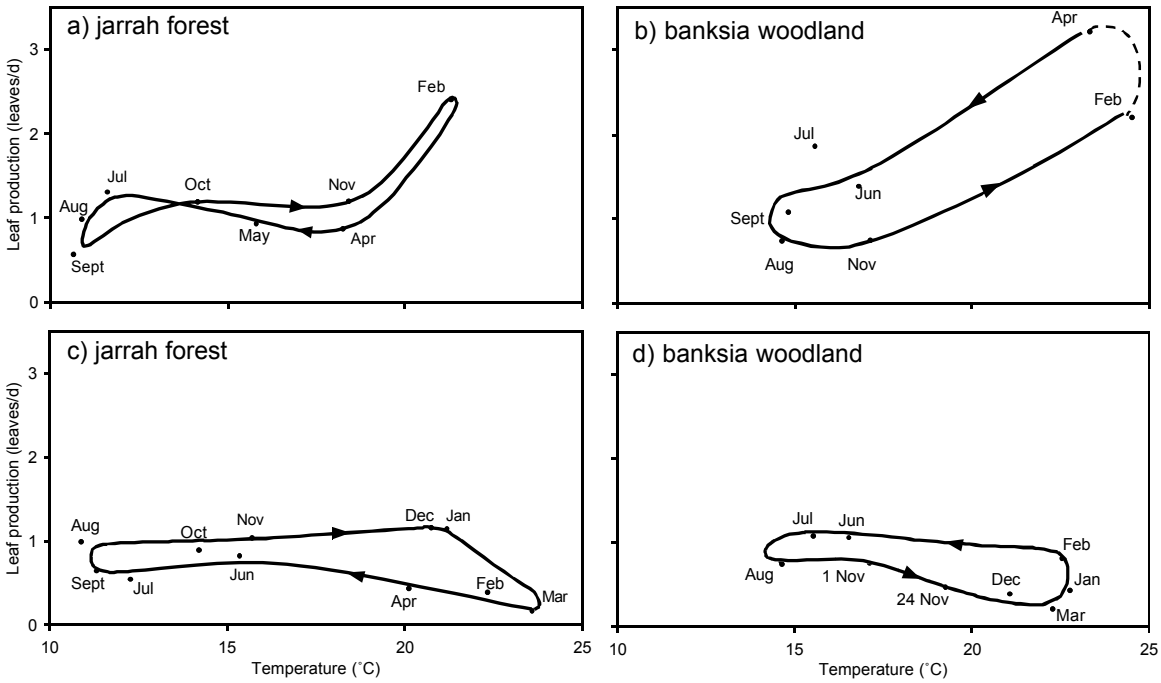

Fig. 2.13 Mean daily temperature-response curves for *X. preissii* leaf production throughout the year. Curves represent periods when water in the (a) jarrah forest and (b) banksia woodland is not limiting, and when water in the (c) jarrah forest and (d) banksia woodland is limiting. Data have been pooled for 2.3 years (1998–2001), and leaf production is given as the mean of six grasstrees from each measurement period.

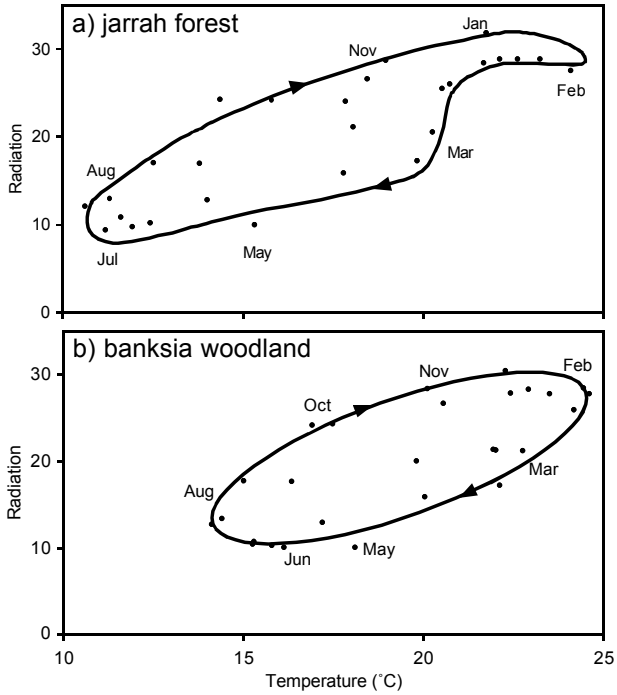

Fig. 2.14 Relationship between incident solar radiation (mJ m^{-2} d^{-1}) and mean daily temperature during the year, for the (a) jarrah forest and (b) banksia woodland. Data are the mean for the measurement periods used in Fig. 2.13.

2.3.1.2 Seasonal patterns of leaf elongation and death

Rates of leaf elongation (progression from one size category to the next) and leaf death for *X. preissii* from both the jarrah forest and banksia woodland are given in Fig. 2.15a,b. Leaf elongation follows a seasonal pattern. In jarrah forest the rate at which young leaves progressed to the intermediate category was highest during the growing season starting in late winter (August-September), when mean daily temperature is about 12 ˚C, and ceasing during the hottest summer period, January/February. Young leaf elongation of the banksia woodland grasstrees did not have the somewhat undulating annual pattern as those in the jarrah forest. The woodland plants appeared to have a shorter growing season, peaking then subsiding earlier than for leaf production. In April (autumn) 1999 these grasstrees showed a surge of young leaf progression (Fig. 2.15b) coincident with a single heavy rainfall event of 50.2 mm, resembling the summer leaf production/rainfall response described above in Section 2.3.1.1. Young leaf elongation for both habitats during the particularly dry 2000/01 growing season (Fig. 2.1) was not substantial.

The intermediate leaf category contained a range of leaf sizes, from very short to almost mature, representing the largest increase in leaf size. Intermediate leaves progressed most rapidly during mid-summer, 1998/99 and 1999/00, in the jarrah forest. Intermediate leaf elongation during the 2000/01 growing season was slightly out of phase with the previous years, reaching a marginal peak in spring. Aside from the 2000/01 growing season and a noticeable increase in intermediate leaf elongation outside of the growing season (late autumn/winter) during 1999, intermediate leaf growth tended to follow the earlier young leaf growth for the jarrah forest grasstrees. Similarly, banksia woodland grasstree intermediate leaf growth also tended to most closely reflect young leaf growth patterns, with the exception of the dry 2000/01 growing season where all growth was low.

Leaf age at maturity was not significantly different for unburnt grasstrees in both habitat types ($P = 0.729$, repeated-measures two-way ANOVA): ranging from 1.3 to 1.6 years (Fig. 2.16). However, the season in which grasstree leaves were initiated affected leaf age at maturity ($P = 0.015$, repeated-measures two-way ANOVA), independent of habitat (habitat × season, $P = 0.430$): leaves produced in summer appeared to reach maturity at a younger age than those produced in the other seasons (Fig. 2.16).

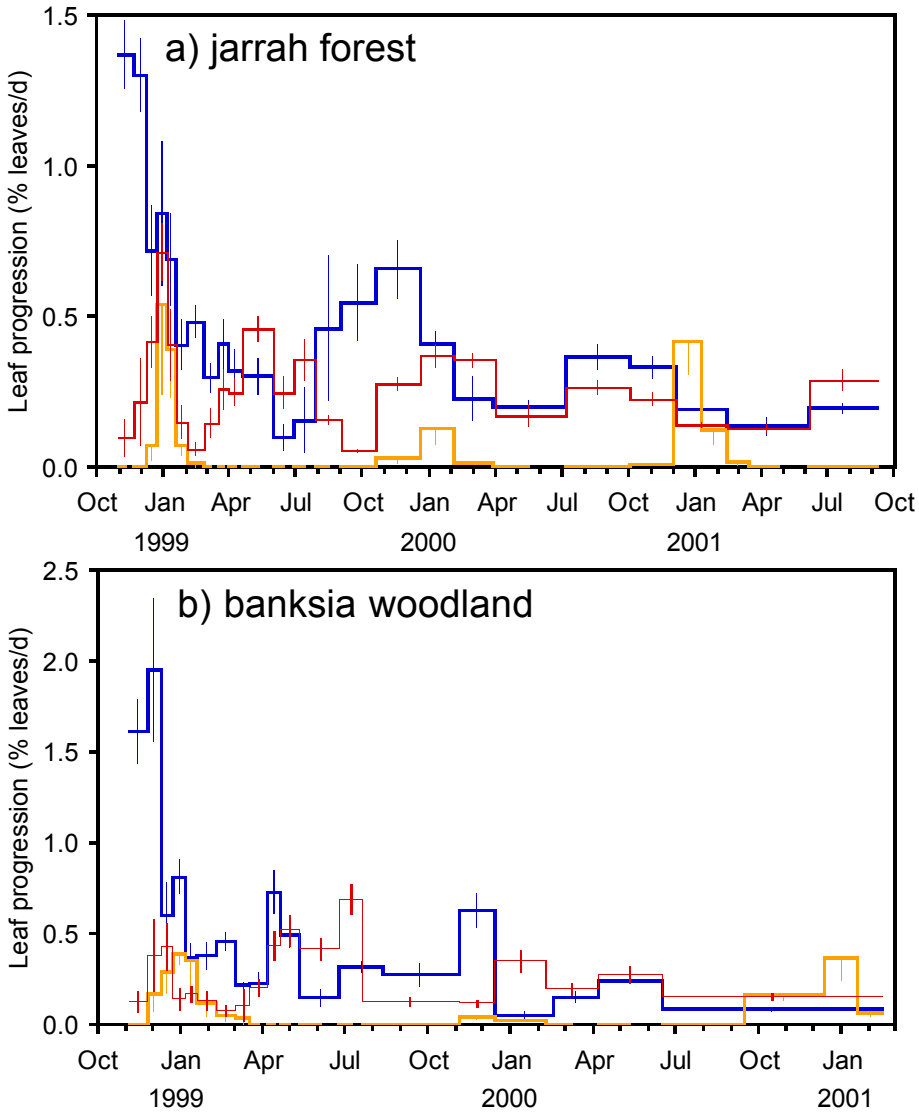

Fig. 2.15 Leaf elongation and death of *X. preissii* growing in (a) the jarrah forest and (b) banksia woodland, from 1998 to 2001. Leaf elongation is expressed as two phases of leaf development: 1) the percentage of young leaves that shifted to the intermediate leaf category per day (—), and 2) the percentage of the intermediate leaves that shifted to the mature leaf category per day (—). Similarly, leaf death is the percentage of mature leaves that died per day (—). Data are the means of six plants for each measurement period (horizontal line) ± SE (SE is only indicated in one direction for leaf death to reduce interference).

Figures 2.15a,b demonstrate the regularity in the timing of annual leaf death for grasstrees from both habitats. Leaf death only occurred during summer, overlapping with the annual peak in leaf production and elongation. During the summer of 1999/00 leaf death was lower than for the other two years monitored; maximum rate of leaf death was three times lower for the jarrah forest and nine times lower for the banksia

woodland grasstrees than other years. Total rainfall over that summer period and for the preceding spring was greater than for the other years. Leaf age at time of death was similar for both habitats: jarrah forest grasstree leaves died after a mean period of 2.25 ± 0.05 years (n = 2) and banksia woodland grasstree leaves died after a mean period of 2.18 ± 0.02 years (n = 6). The mean total number of leaves that died per grasstree (n = 4) during the 1999/00 summer was 582 ± 125 in jarrah forest and 463 ± 84 in banksia woodland. In comparison, the mean total number of leaves produced per grasstree (n = 4) during the year July 1999 to July 2000 was 589 ±82 for jarrah forest and 434 ± 67 for banksia woodland. Leaf production was not significantly different from leaf death in the jarrah forest and banksia woodland (P = 0.9051 and P = 0.3946 respectively, paired t-test).

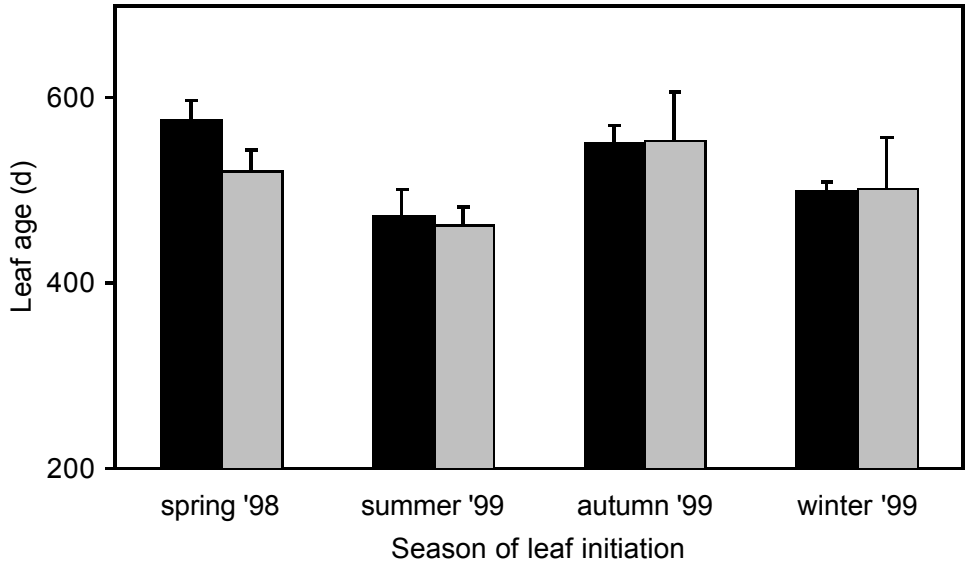

Fig. 2.16 The age of *X. preissii* leaves at maturity in jarrah forest (■) and banksia woodland (■), and identifying the season in which they were produced. Data are the means of six plants ± SE.

2.3.2 Root growth

Three characteristics of new roots (number per grasstree, total biomass per grasstree, and root size) were quantified to interpret and explain *X. preissii* root growth phenology. Seasonal changes in these characteristics reflect changes in the vigour of root production and growth. Simultaneously, the process of new root maturation or desiccation resulting in the decay of the cortex and attainment of the 'old root' structure, influenced the number of new roots identifiable, and subsequently the data collected. Separating the processes of new root growth and new root decortication, so that root growth might be

measured independently, was not possible. However, as root cortex deterioration generally occurred during dry summer conditions, which were negatively correlated with root growth (see further discussion of results), the net effect of this process on the data presented here would not alter root growth seasonality.

New roots commonly emerged in clumps forming an intermittent ring around the caudex, at a depth marked by the shallowest pre-existing roots (20–30 cm). Growing root tips were encased in a heterogeneous sheath, consisting of secreted mucilage and sand grains, the evidence of which was still apparent on the older proximal portions of the roots. New root production appeared to commence in winter, evident by a 73% increase in the number of grasstrees with new roots from autumn to winter (Table 2.3). However, from the results of the binomial test, the proportion of grasstrees excavated in winter that had new roots was not different from 50% (no seasonal effect). This result seems surprising after examining the raw data, and small sample size is likely to have prevented support for the alternate hypothesis (ie. probability of grasstrees bearing new roots > 0.5). Statistically, it is in spring that new roots were most likely to be present (Table 2.3). All root characteristics were significantly different across the four seasons from winter to autumn (Kruskal-Wallis test, Table 2.4). Also, all root characteristics were greatest during late-spring pointing to an annual peak in root growth, supported by a significant difference in root size between winter and spring (two-tailed Mann-Whitney with sequential Bonferroni correction, Table 2.4). Concurrent with new root recruitment during November 2000, older roots of *X. preissii* were observed to continue growing from their tips, often branching at this point (Fig. 2.17). The significant decrease in the number of new roots per grasstree and the total new root biomass, from spring into summer (one-tailed Mann-Whitney with sequential Bonferroni correction, Table 2.4), appeared to mark the end of the root-growing season. The results of the binomial test were unable to support this. However, these results do demonstrate that the probability of finding new roots in the following season (autumn) is less than 50% (Table 2.3). While spring supported peak root growth, root characteristics (Table 2.4) generally pointed to an annual low in autumn (May), where only 10% of grasstrees exhibited new roots. Despite this, in all seasons, severing the wiry stele of most old roots could reveal moist, living, porous tissue.

Table 2.3 The number of grasstrees growing in the banksia woodland with new roots during each season. The number of grasstrees with new roots (n_2) is expressed as a percentage of the total number of grasstrees excavated in each season (n_1). For each season, the probability (p) that a grasstree will bear new roots was analysed using a one-tailed binomial test. To obtain an overall p for the distribution, it was assumed that there is no root growth in summer-autumn and all plants have new roots in winter-spring from which the average p is 0.5. Thus, H_O: $p = 0.5$ was used for all tests, while H_A: $p > 0.5$ was used for winter and spring, and H_A: $p < 0.5$ was used for summer and autumn. An outcome statement indicating the probability of finding new roots (p) is given in parentheses, based on the results of each test.

Site	Season	n_1	n_2	% grasstrees with new roots	Binomial test (P-value)
YC	winter	6	5	83	0.109 ($p = 0.5$)
	spring	5	5	100	0.031 ($p > 0.5$)
	summer	5	4	80	0.188 ($p = 0.5$)
	autumn	10	1	10	0.011 ($p < 0.5$)

Table 2.4 A summary of *X. preissii* new root characteristics during each season (August 2000, November 2000, February 2001 & May 2001). Estimated total new root mass, number of new roots per grasstree and size of new roots, are means ± SE of the number of plants with new roots (n). Lower case letters indicate the significant differences between results of adjacent seasons for Mann-Whitney test (with sequential Bonferroni correction) for new root characteristics obtained from Kruskal-Wallis test (P-values shown). Similarly, upper case letters indicate the significant differences between autumn and winter.

Site	Season	n	Total root mass /grasstree (g)		No. of new roots/grasstree		Root size (g/cm)	
YC	winter	5	11.7 ± 1.4	a A	14 ± 2	a A	0.05 ± 0.01	a A
	spring	5	42.3 ± 9.7	a	24 ± 8	a	0.13 ± 0.04	b
	summer	4	1.6 ± 0.5	b	4 ± 1	b	0.07 ± 0.00	b
	autumn	1	0.4	b A	2	b A	0.07	b A
P-value			0.009		0.021		0.013	

Fig. 2.17 Two mature *X. preissii* roots in late spring 2000. Branching from behind a rotten apical meristem (left), and multiple branching behind the terminal meristem which itself has bifurcated forming new apical meristems (right). Scale in cm.

Of the new roots excavated in each season, not all were in a state of active growth (Fig. 2.18). The long, tapered, yellow/green tipped new roots (Fig. 2.3) suggested active elongation, and were present predominantly during winter and spring. 50% of roots growing in autumn have growing tips. However, this is based on one grasstree that bore

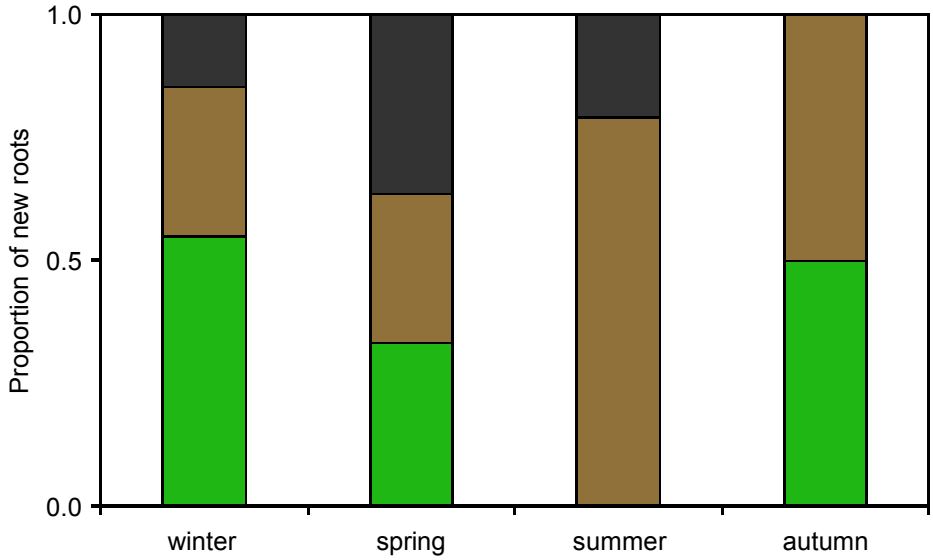

Fig. 2.18 Seasonal variation in the proportion of grasstree new roots allocated to each of three categories of root tip condition. The three categories were: 1) growing (■), 2) dormant (■), and 3) rotten (■) (see Fig. 2.2 for detail of these conditions). Roots were excavated during each season from 2000 to 2001 (August, November, February and May). Proportions are the mean of 4–5 plants (see n_2 in Table 2.3), except for autumn, which was based on root sampling from a single grasstree.

only two new roots. New roots in summer were predominately dormant, characterised by a stunted black tip (Fig 2.3), and further supports the notion that summer marks the end of the growing season. The mean number of new roots per grasstree during spring was counter-balanced by an annual high proportion of these roots having rotten tips (36%). As a result, the number of actively growing new roots present during spring was reduced to 8 per grasstree, the same as during winter, when tip rot was less prevalent. Tip rot appeared to be associated with the presence of a parasitic scale insect in the family *Margarodidae* (Melinda Moir; Environmental Biology, Curtin University of Technology), distinguished in Figure 2.3 as pale pink oval structures on the root surface.

Grasstree roots were estimated to extend vertically to a depth of at least 6.4 m, growing on leached sand over limestone on the Swan Coastal Plain. These roots were constricted into gaps in the limestone as a pathway through the hard substrate (Fig. 2.4).

2.3.3 Grasstree water relations

2.3.3.1 Seasonal water potentials

Predawn xylem water potential (Ψ_{PDX}) for jarrah forest grasstrees reached an annual low of about −1.0 MPa during mid-autumn (Fig. 2.19a), but rarely fell below this level. The annual low for the banksia woodland grasstrees was during summer (Fig. 2.19a), and the lowest recorded Ψ_{PDX} for an individual plant was −1.26 MPa. At no point did Ψ_{PDX} for grasstrees from either habitat reach their respective mean Ψ_{TLP} (mid-autumn): −1.85 ± 0.47 MPa for jarrah forest and −2.18 ± 0.17 MPa for banksia woodland. Ψ_{PDX} was highest (less negative) during mid-spring to early summer for the jarrah forest grasstrees, while Ψ_{PDX} for the banksia woodland grasstrees peaked in late winter/early spring. During this period it was not unusual to record potentials of 0 MPa.

Between years, maximum annual Ψ_{PDX} recorded during spring was consistent, whereas minimum Ψ_{PDX} showed more variability, with higher Ψ_{PDX} for the summer/autumn of 2000 in both habitats, relative to other years. An unseasonal rise in Ψ_{PDX} during the autumn of 1999 for grasstrees in jarrah forest and banksia woodland was correlated with a significant single rain event (Fig. 2.1), the same event that triggered an increase in leaf production and leaf elongation. Midday xylem water potential (Ψ_{MDX}) had a similar seasonal pattern to Ψ_{PDX}. However, in the jarrah forest the curves were out of phase with

maximum and minimum potentials recorded earlier (late winter and mid-summer respectively, Fig. 2.19b).

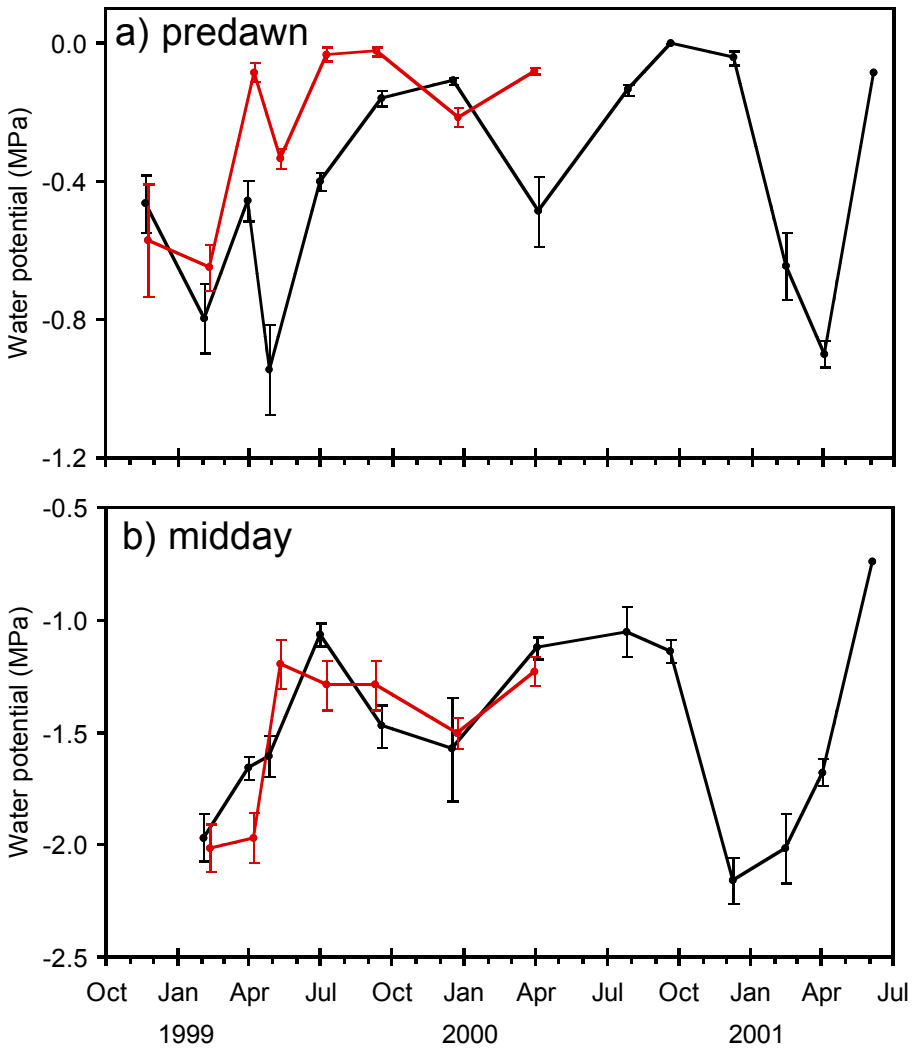

Fig. 2.19 Predawn (a) and midday (b) xylem water potential for *X. preissii* in jarrah forest (●) and banksia woodland (●) between 1998 and 2001. Data are means of six plants ± SE.

2.3.3.2 Response to simulated summer rain

Prior to the application of water to simulate summer rain, grasstree Ψ_X reflected the annual low values described above (Section 2.3.3.1), typical of summer/autumn. Measurement of predawn soil water potential (Ψ_{PDS}) was hampered by irregularities in the performance of the buried sensors, which required that the accuracy of all measurements be closely scrutinised. Ψ_{PDS} for all soil depths during the pre-watering period was considerably lower (more negative) than grasstree Ψ_{PDX} and Ψ_{MDX} (Table 2.5). Immediately following watering, both Ψ_{PDX} and Ψ_{PDS} increased (Table 2.4). The

change in Ψ_{PDX} and Ψ_{MDX} (24 h after watering) was significant (Table 2.5). Ψ_{PDX} (< 24 hours after watering) was not significantly different from Ψ_{PDS} at 33 cm depth, but it was significantly different from Ψ_{PDS} at 43 cm depth (Table 2.5).

Table 2.5 Soil water potential (Ψ_S in MPa) and xylem water potential (Ψ_X) measured in banksia woodland (YC) during summer/autumn of 2001. Three measurements were made prior to watering (water application indicated by a horizontal broken line) and once less than 24 h after watering. Ψ_S was recorded predawn at four depths at four locations. Ψ_X was measured on six grasstrees, predawn and midday. Data are means ± SE and *n* is given in parentheses. Lower-case letters indicate significant differences across dates for adjusted (Bonferroni) pairwise comparisons for Ψ_X obtained from repeated-measures ANOVA (*P*-values shown). Upper case letters indicate the significant differences across soil and grasstree leaf results for Tukey's analysis for post-watering water potentials obtained from one-way ANOVA (*P*-value shown). Transformed data were analysed, but untransformed data are shown. For both analyses A and a are highest values.

Date	Ψ_S (predawn)				Ψ_X (n = 6)	
	13 cm	23 cm	33 cm	43 cm	Predawn	Midday
					$P = 0.005$	$P < 0.0005$
January	-3.8 (1)	-4.1 ±0.1 (3)	-3.6 ±1.0 (4)	-3.3 ±0.8 (4)	-0.6 ±0.1 a	
February	-5.6 ±0.4 (4)	-5.5 (1)	-5.1 ±0.5 (4)	-4.3 ±0.7 (4)	-0.7 ±0.2 ab	-1.0 ±0.2 a
20 April		-1.8 (1)	-2.0 (1)	-2.3 (1)	-1.1 ±0.2 b	-1.9 ±0.1 b
21 April	-0.4 (1)	-0.1 (1)	-1.0 ±0.2 (3)	-1.2 ±0.6 (3)	-0.7 ±0.1 a	-0.8 ±0.1 a
ANOVA $P = 0.019$			AB	B	A	

Leaf production demonstrated an equally dynamic response to watering during autumn drought (Fig. 2.20). Prior to watering, the site received a total of 6.5 mm of natural rain (over 92 days) and grasstrees experienced the lowest recorded summer leaf production rate since my project commenced. Less than 24 h following watering the mean leaf production rate increased 7.5 fold, and within 5 days of watering the new rate had doubled. Nineteen days after watering, leaf production rate began to fall, despite an extra 32 mm of natural rainfall. Of note is the considerable increase in standard error that accompanied the increase in leaf production.

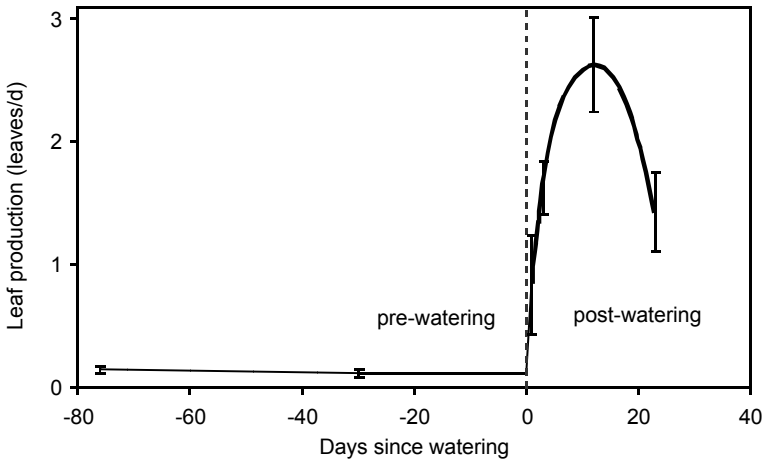

Fig. 2.20 Effect of artificial watering, simulating 59.2 mm of rainfall, on leaf production by *X. preissii* in the banksia woodland. Data are means of six plants ± SE.

2.3.4 Indirect measurement of leaf production

Some difficulty was encountered in distinguishing the boundary between annual bands on the stem of grasstrees in both habitats. In jarrah forest, rot and termite damage to the thatch of some grasstrees had caused the associated dead leaf bases to decay and crumble, resulting in the loss of the unique colours of the seasonal bands that highlight annual growth bands. *X. preissii* in the banksia woodland, although unaffected by rot, displayed poor colour distinction between seasonal bands. The mean number of leafbases counted per annual band on jarrah forest grasstrees was less than the mean number of leaves produced in one year by the same plants, and the reverse was true for grasstrees in the banksia woodland. A two-way ANOVA revealed that the counts obtained using the two methods were not significantly different, and no difference existed between the results from the two habitats (Table 2.6).

Table 2.6 Comparison of results from two methods of determining mean annual leaf production of *X. preissii* in two contrasting habitats: direct count of the number of leaves produced in two years, and a count of the number of leafbases contained in two annual growth bands. Data are means for six plants ± SE.

	Jarrah forest	Banksia woodland
Direct leaf count	506 ± 75	476 ± 23
Leafbases per annual band	455 ± 31	519 ± 47
Two-way ANOVA (*P*-values)		
Habitat	0.720	
Method	0.937	
Habitat × method	0.330	

2.4 Discussion

Conventional methods used to measure vegetative growth in dicotyledenous trees and shrubs, eg. height, girth, shoot extension, are impractical for use on grasstrees. The technique for quantifying leaf production used here was devised as a surrogate for these traditional methods. The easy accessibility of the foliage and growing apex, the responsiveness of leaf initiation to the environment, and the accuracy, unobtrusiveness and simplicity of the method made it suitable for monitoring the subtleties of grasstree growth. The ability to apply this method immediately following fire-removal of the foliage was another advantage (see Chapter 3 for details).

Unlike most plant species outside the Tropics (Lieth 1974, Fritts 1976), *X. preissii* does not have a discrete growing season, undergoing leaf initiation and elongation throughout the year, despite the natural climatic extremes experienced within its distribution. Like *X. preissii*, the grasstree *Kingia australis* (family Dasypogonaceae, Rudall & Chase 1996) on the Darling Scarp produces leaves throughout the year (Lamont 1981). Lamont's data indicate a spring flush of leaf elongation followed by a summer minimum, which is in general keeping with *X. preissii*. Similarly, *X. reflexa* (synonymous with *X. preissii*) in Tutanning Nature Reserve (120 km ESE of Perth) displays continuous foliar growth throughout the year, but with the greatest increments from June to August and October to December (Specht *et al.* 1981). A study of 23 common species often co-occurring with *X. preissii* on the Swan Coastal Plain noted that shoot growth occurred in one or more of the species in all seasons except summer-early autumn (Bell & Stephens 1984). Species with a spring-summer growth rhythm continued vegetative growth until the watertable level fell below the clay horizon, after which dormancy followed. In another study, the same *Banksia* spp., dominant at my banksia woodland sites (Table 1.1), grew rapidly during early summer, but showed no growth during mid- to late-autumn (Lamont & Bergl 1991). Lamont and Bergl (1991) did, however, show that drought was avoided by prolonged access to water stored at depth and control over its use. Similarly, a study of the water relations of jarrah forest overstorey and understorey (including *X. preissii*) demonstrated that plants within this habitat remain physiologically active during the summer (Crombie 1992). In light of these observations, what appears to make *X. preissii* special is its ability to continue growing throughout the year, albeit at a reduced rate during the driest period.

As shown in my study, growth of roots is considerably more difficult to measure than that of the foliage (Böhm 1979, Rorison 1981), and accounts for the comparative lack of studies considering both aerial and subterranean vegetative growth phenology. Kummerow *et al.* (1981) concluded from a review of root studies in summer dry areas, that root systems in mediterranean climate regions are plastic in their response to environmental conditions. Hence, although my study provides an insight into root growth by *X. preissii* in banksia woodland, the results may vary somewhat from the same species growing in the jarrah forest, providing the opportunity for a physically challenging, comparative study. Unlike leaf growth, *X. preissii* exhibits a well-defined season of root growth on the coastal plain, commencing in winter and finishing during summer. The related grasstree *Kingia australis* generally initiates root primordia in winter. However, unique to this species, these roots originate from the stem apex (Lamont 1981), and not from a deeply-seated rootstock or caudex as with the *Xanthorrhoea*. This difference in the environment immediately surrounding the roots may explain the continued root elongation through summer by *Kingia australis* (Lamont 1981), long after *X. preissii* has terminated root growth.

Regressing climatic data against leaf production rates was one method employed to interpret the leaf production results. However, the final relationships developed only accounted for 25–45% of total variance. Nevertheless, the results did give an insight into the likely relationship between growth of *X. preissii* and climate. The inclusion of microsite climatic data, in an attempt to match the plant specific scale used in measuring leaf production, would be unlikely to alter the relationships, as for jarrah forest grasstrees both modelling attempts indicated the possible importance of daylength in predicting leaf production. However, it is also likely that the strongly seasonal pattern of leaf growth may be correlated with a number of other seasonally-predictable variables providing equivalently low r^2 values. The specific use of daylength for modelling jarrah forest grasstree leaf production instead of temperature was based on a marginal difference in r^2 values. In my case, daylength offered a means of predicting leaf production, but should be treated as only one possible climatic variable that directly controls leaf production. In banksia woodland, rainfall appears to be an environmental factor that has value in predicting summer growth, but is unlikely to be a factor controlling the timing of major seasonal growth phases. It became evident that, while these modelled relationships have much to offer, caution must be applied in their

interpretation, and for this reason considerable 'weight' has been given to trend analysis and supportive literature in deciphering grasstree phenology.

2.4.1 The switch in growth phase between two seasonally-distinct periods of the year

The alternating pattern of fast then slow leaf growth of *X. preissii* can be described as sinusoidal, reflecting the hot/dry summer season and then cool/wet winter season that dominates the bimodal mediterranean climate. During the transition between the annual climatic extremes *X. preissii* switches from one state of leaf growth to the other, comparable to the change from active growth to dormancy (and vice versa) that some species experience under harsh climatic conditions (Ackerman & Bamberg 1974, Mohr & Schopfer 1995).

The relatively sudden switch from slow winter leaf production to the fast summer flush in *X. preissii* suggests that an environmental trigger or threshold may be involved. From predominantly northern-hemisphere examples, it is accepted that a period of low temperature is required by winter-dormant plants to initiate active growth (Flint 1974), and often, prolonged exposure to a subsequent higher temperature threshold is also needed (Perry 1971). More applicable to *X. preissii*, research has identified lower temperature thresholds for growth of the vegetation of southern Australia, below which growth is inhibited or ceases altogether (Specht & Rayson 1957a, Groves 1965, Specht 1973, Specht *et al.* 1981). For dominant shrub and tree species, these temperature minimums fall in the range of 16–18 ˚C. From the relationship between mean daily temperature and leaf production (Fig. 2.13a,b), *X. preissii* was observed to exhibit a positive response once the temperature exceeded 16.5–17.5 ˚C. However, there were no records for the crucial period around late November/early December when the fast-growing-season starts (data were restricted to those where water was not limiting). As a consequence, this minimum may be an underestimate and a value closer to 20 ˚C, suggested by trends in leaf production (Fig. 2.6), may be more accurate. Strictly speaking, because *X. preissii* grows continuously, the minimum temperature of 20 ˚C marks the change from slow to fast growth, not a transition from an inactive state to one of active growth, that the minimum threshold of 16–18 ˚C represents for other Australian species. Therefore, comparison of these minima must be treated cautiously.

While 20 ˚C, as the threshold minimum for fast leaf production seems high, the rate of leaf elongation, although not as seasonally consistent, generally increased in late winter/early spring, coincident with temperatures around 12 ˚C. The location of the apical meristem could offer an explanation for this substantial difference between minimum temperatures for leaf production and elongation. Unlike tree and shrub components of the mediterranean flora, *X. preissii* produces leaves from a single well insulated apical meristem. As a consequence, it is likely that while temperatures rise during spring the meristem remains cooler than the surrounding elongating leaves, delaying its growth. Alternatively, with the start of the fast-growing-season nutrients and photosynthates may initially be directed to the large number of pre-existing immature leaves. Therefore, despite the minimum temperature for leaf production being reached, negligible growth was observed.

Specht & Specht (1999) view upper and lower temperature thresholds as important controls of plant seasonal phenophases, and considered them to fall 5 ˚C either side of the optimum. Optimum temperatures for vegetative and reproductive growth generally lie between 20–30 ˚C (Bell & Stephens 1984). Peak leaf production occurred at 25.5 ˚C for jarrah forest grasstrees and 23.3 ˚C for banksia woodland grasstrees, this equates to a maximum range of suitable leaf production temperatures from 18.3–30.5 ˚C. While this maximum threshold seems reasonable, my data clearly show that this minimum threshold is inaccurate, as *X. preissii* can grow at temperatures substantially less than 18.3 ˚C.

The inability of the first regression model for the banksia woodland to predict the start of the 1998/99 growing season (Fig. 2.11b) supports the idea that growth is initiated by a more complex trigger than a simple positive relationship with temperature, as assumed in the model. Furthermore, the same model over-predicts the leaf production response during October 1999, where conditions (water and temperature) were not sufficient to stimulate *X. preissii*. Experimental testing of these two ideas under controlled conditions would be physically impossible using mature plants, and the value of generalising from seedling trials is questionable given the subterranean existence of the seedling apical meristem (Koch & Bell 1980, Staff & Waterhouse 1981, Gill 1993). It should be noted that reproductive growth (inflorescence production) of *X. preissii* occurs from winter to spring (July–November, Marshall 1986), prior to the flush in leaf production in late

spring. This suggests that the mechanism controlling the start of fast summer leaf production is not due to a physiological restriction to growth generally at lower temperatures.

Root growth in mediterranean Australia often precedes foliage growth (Lamont 1976, Specht *et al.* 1981, Lamont & Bergl 1991, Specht & Specht 1999), commencing in winter or spring. Similar findings for more temperate species (Kozlowski 1971, Hoffman 1972) have been explained as a consequence of roots having lower temperature minima (Hoffman 1972). Root production in *X. preissii* was first measured during mid-winter (August), but it is likely that growth would have begun sometime earlier, after soil moisture recharge from the initial winter rains. Although this change in soil moisture could be a direct trigger for the commencement of elongation of existing roots, the production of new roots from the caudex would require an internal stimulus. Such a stimulus may involve an increase in water potential, or the effect of net movement of nutrients and photosynthates towards the now active mature root tips, via the base of the caudex. The peak of new root production in *X. preissii* occurred in spring, indicating that root growth vigour is partly dependent upon temperature. The combination of adequate soil moisture and rising temperatures during spring was not only conducive to root growth but also seemed to encourage the infestation of growing root tips with parasitic scale insects. By the end of summer, new root growth had succumbed to high water deficits, and the majority of new roots displayed the characteristic dark suberised tip resulting from metacutisation (Wilcox 1954).

For a number of *Hakea* species growing under the mediterranean climate of south-western Australia, drought-induced root dormancy can be broken by the application of water (Lamont 1976). Considering the timing of new root production in *X. preissii*, it seems that the late start to the foliar growing season may reflect a timing that avoids competition between the roots and shoots for photosynthates and other essential resources required for growth. Experimental pruning of apple and plum trees, stimulating intense shoot growth, revealed a negative effect on new root production, suggesting competition between roots and shoots for nutrients when reserves were not adequate at times of rapid utilisation (Head 1967).

After the break of summer drought by the winter rains, *X. preissii* undergoes the equivalent reverse of what has been discussed up to now: a seasonal reduction in growth rate. The cessation of summer growth and subsequent induction of a dormant state noted in other species provides a fairly loose analogy with the observed slowing of grasstree growth during the transition from summer to winter. Dormancy induction is a process controlled by many environmental factors (Perry 1971, Flint 1974). Although photoperiod is regularly cited as a key agent controlling dormancy induction, this does not appear to be the case with most components of the Australian flora (Specht & Brouwer 1975, Paton 1978). Additionally, its effect can be overridden by temperature, soil moisture, substrate nutrient concentrations and photoperiod light intensity (Perry 1971). Consistent with this conclusion is the greater difficulty in explaining the timing of grasstree growth response to seasonal changes during autumn. *X. preissii* tends to switch from rapid to slow leaf production near the end of May. The predictability of this timing suggests that equally predictable climatic factors are responsible, such as daylength or air temperature. Jarrah forest daily mean temperatures in May are similar (15.0 ˚C) to Groves' (1965) temperature threshold of 15.6 ˚C, above which heath vegetation at Wilson's Promontory in Victoria actively grew. Milder temperatures in banksia woodland (compare Fig. 2.1a,b; mean daily temperature for May was 17.2 ˚C, and 15.9 ˚C in June) may account for a slightly extended growing season in this habitat.

Leaf growth characteristics during autumn 1999 provide an exception to this generalisation. The onset of early rains in autumn has the potential to release *X. preissii* from a growth pattern tightly restrained by water availability, before the plant is forced into a winter mode of growth. As a consequence of significant rain in late autumn, leaf growth escalated for banksia woodland grasstrees (and to a lesser extent grasstrees in jarrah forest), resulting in an extended growing season. Growth was apparently prolonged prior to dormancy by ensuring good moisture and nutrient supplies (Zahner *et al*. 1964). The inability of the temperature/rainfall regression model to predict the elevated growth of banksia woodland grasstrees during July may be explained by the complex interaction of controlling factors. Predicting leaf production during the summer/winter transition period discussed here exposes a definite deficiency in the second banksia woodland model (best $r^2 = 0.459$). It is obvious that the use of a constant may not always be appropriate to account for the potential variability in leaf production that can occur during this period. This point is justified by the poor performance of the

second jarrah forest grasstree regression in predicting nine months of data, independent of those used to derive the model.

The change in grasstree growth characterising the period leading up to summer seems primarily controlled by temperature, not unlike the phenomenon of vernalisation. Yet the transition from autumn into winter is somewhat more complex, undoubtedly reflecting the adaptation of *X. preissii* to a climatically unpredictable time of the year associated with its distribution.

2.4.2 The role of temperature in the possible origin of grasstree phenology

By substituting leaf production for relative shoot growth, the leaf production response of *X. preissii* to temperature (Fig. 2.13a,b) can be directly compared with overstorey mesotherms (≅ subtropical; *Eucalyptus* and *Banksia* spp.), and understorey microtherms (≅ warm temperate; *Allocasuarina pusilla*, *Leptospermum myrsinoides*) in the mediterranean region of Australia (Fig. 2.21a,b from Specht & Dettmann 1995). When water is not limiting, *X. preissii* responds to a range of temperatures most similar to that of the mesotherm overstorey species. In the growing season, from late spring through to autumn, leaf production increased with rising temperature (Fig. 2.13a,b), but as the full temperature range for growth did not occur during my study, the observed leaf production pattern resembled the truncated bell-shaped curve of mesotherm overstorey species (Fig. 2.21a). Continuous growth of *X. preissii* during the cool winter tends to contradict the classification of *X. preissii* as a mesotherm according to Specht and Specht (1999), who classify mesotherms as those plants exhibiting a growth response between 15–25 ˚C. Although its growth characteristics at higher temperatures are consistent with a subtropical rhythm, growth performance at lower temperatures indicate that *X. preissii* can function under an unusually broad range of temperatures. The thermal definition of a mesotherm by Nix (lower threshold 5 ˚C and upper threshold 33 ˚C, 1982) is more appropriate; adequately explaining why *X. preissii* as a mesotherm can grow constantly over the annual range of temperature recorded during this study (10–26 ˚C). In the banksia woodland where the canopy is naturally sparse (15% canopy cover at YC, unpublished), it is not surprising that *X. preissii* behaves more like an overstorey species than an understorey species. However, *X. preissii* also exhibits similar leaf growth patterns in the jarrah forest, where the overstorey is considerably denser (73% at

MC, Chapter 5). Following Specht's argument that it is the upper-stratum species that have retained a subtropical (mesotherm) growth rhythm, this suggests that *X. preissii* (likely to be a relatively old species) would have originated from an open habitat during the subtropical early Tertiary and more recently radiated to habitats with more complete canopies such as the jarrah forest.

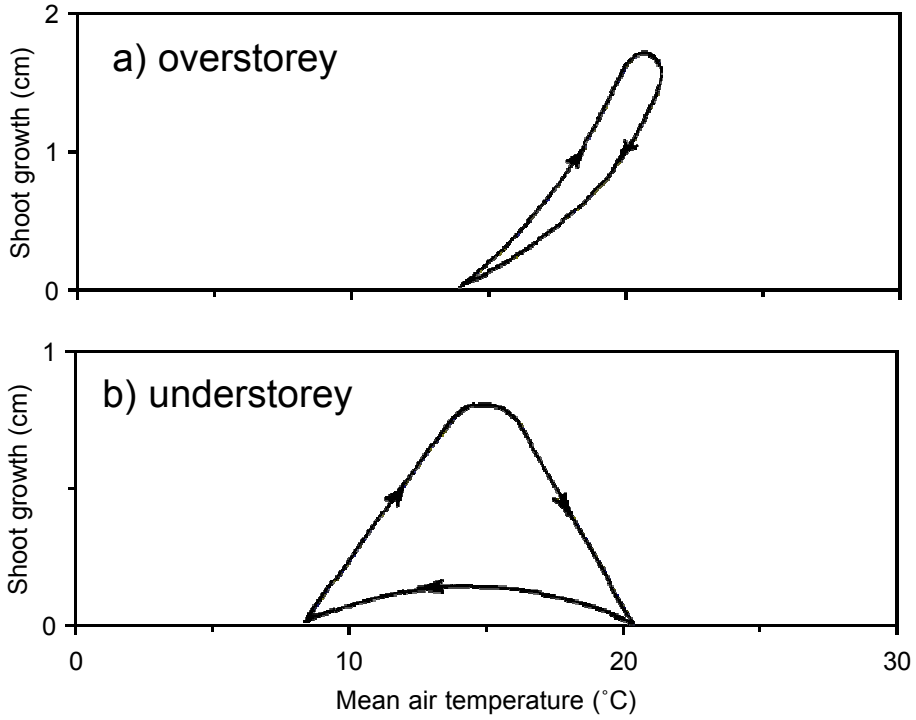

Fig. 2.21 Relative growth increment of tagged shoots of upper-stratum species in the mediterranean region of Australia (Keith, South Australia). (a) Overstorey mesotherm *Eucalyptus* and *Banksia* spp. on nutrient-poor to average nutrient soils, and (b) understorey microtherms *Allocasuarina pusilla* and *Leptospermum myrsinoides* on nutrient-poor soil (from Specht & Dettmann 1995).

For a palm-like species, the crowded leaf arrangement and leaf size of *X. preissii* could be viewed as a moderately intricate crown structure, that Specht and Dettman (1995) associate with the microtherm growth rhythm of understorey species (see Introduction), not the mesotherm rhythm retained by this grasstree. Despite the leaves approaching the size of mesophylls (as described for the closely related species *X. australis*. Specht & Rayson 1957a), in comparison with other related genera from more temperate climates that have broad strap-like leaves, such as the cabbage trees (genus *Cordyline)* in New Zealand, grasstrees in the genus *Xanthorrhoea* have comparatively fine, narrow leaves. Adding to this, the presence of sunken stomata, thick cuticle and mesophyll, and much

fibrous tissue (Staff & Waterhouse 1981, Fahn 1990, Shivas *et al.* 1998) suggest an evolutionary response to water stress (Lamont *et al.* 2002). The most likely appearance of these xeromorphic features in the genus would have occurred while exposed to increasing water deficits on the outer boundaries of the distribution of subtropical vegetation contracting with increased aridity during the late Tertiary-Pleistocene (Hopper *et al.* 1996). In light of this understanding, leaf properties and crown structure of grasstrees may have undergone considerable evolutionary modifications in combating water stress, but, evidenced by the retention of a subtropical growth rhythm, do not reflect selective pressures to increase the boundary layer hypothesised for understorey species (Specht & Dettmann 1995).

With the increasing aridity of the Australian continent during the Pleistocene (starting 1.7 million years ago. Lamont *et al.* 1984, Hopper *et al.* 1996) it seems reasonable that water availability would have played the greatest role in shaping the mediterranean vegetation. From this perspective, the gradient from ground stratum species yielding many small, densely packed leaves through to the upper stratum yielding fewer large leaves (Specht & Specht 1999, South Australian heathland) may reflect a parallel gradient of relative water stress (eg. Donovan & Ehleringer 1994). Perennial flora in the ground stratum with shallow root systems are under the greatest selective pressures imposed by water availability, while the overstorey species with large roots penetrating to deep stored water are less affected. The intricate structure of the understorey would therefore represent an adapted mechanism for water conservation, compensating for a very limited root system. The fact that leaf morphology and crown structure does not appear to have evolved to this extent in *X. preissii* indicates that its medium root system (Crombie *et al.* 1988) is adequate to support summer growth. Bell and Stephens (1984) working on Western Australian mediterranean flora concluded that the adaptive significance of the different timing of shoot growth and flowering among this vegetation was related to root depth and availability of soil moisture, complementing the theory of leaf size reduction given here. This discussion has offered a likely explanation for the bias in the evolutionary reduction of leaf size towards understorey species (Specht & Dettmann 1995, Specht & Specht 1999), while offering an alternative theory for the segregation of the timing in shoot growth of overstorey and understorey species. Whether seasonal growth is temperature or water-limited will remain impossible to

unravel among large plants that cannot be manipulated experimentally under controlled conditions.

The truncated hysteresis temperature-response curve for grasstrees in jarrah forest (Fig. 2.13a) can be divided into two six month periods that experience the same range of daily mean temperatures, September to February and March to August. Leaf production is almost identical over the same temperature range for both periods. No seasonally-biased environmental factors appear to be influencing leaf production. Specht and Specht (1999) noted that over the same temperature range foliar growth of *Corymbia (Eucalyptus) intermedia* during spring was greater than during autumn. Greater solar radiation occurs during spring than autumn due to lower cloud cover and to a lesser extent water vapour concentration, aerosols and ozone (Bruce W. Forgan, meteorologist, Australian Regional Instrument Centre, Bureau of Meteorology, pers. comm.). This difference may explain greater spring growth. Marginally greater leaf production occurred during November than for the thermally equivalent period in April for jarrah forest grasstrees, and is consistent with this explanation. Although scant data during summer only permits speculation of the patterns of leaf production over this period, there does not appear to be a seasonal effect of radiation on leaf production at any point during the year.

The reverse trend is apparent for the banksia woodland grasstrees with autumn supporting greater growth, but again this pattern is supported by few data. Slower growth during spring may reflect partitioning of resources away from the foliage during this period, and towards other active meristematic tissues, such as growing roots. The dependency of the temperature-response curve of *X. preissii* on water availability (compare Fig. 2.13a,b with Fig. 2.13c,d) poses a possible flaw in this approach for explaining subtle effects of radiation on grasstree leaf production during thermally-equivalent periods of the year. A simple version of the biological principle of limiting factors states: a biological process, such as growth, cannot proceed faster than is allowed by the most limiting factor (Fritts 1976). The criterion for selecting leaf production data during conditions assumed to be unrestricted by water (vigorous growth observed in conjunction with > 20 mm of rainfall) may still have been inadequate to ensure complete independence from water availability. Therefore the pattern of leaf production described in Figure 2.13b (and for that matter Fig 2.13a) may still reflect water availability. A

review of this criterion to include soil moisture and/or plant water potential measurements may resolve this dilemma.

2.4.3 The importance of water availability

Somewhat neglected to this point in the discussion is the importance water has on the growth patterns of *X. preissii*. Due to a high water deficit during summer/autumn leaf production may be reduced to levels below the winter baseline regardless of daily temperature, and will only recover after sufficient rainfall either from the start of winter rains or unpredictable, episodic summer rainfall. The growth patterns depicted in Figure 2.13a,b represent those data collected during periods not limited by water availability. If all data were included, the relationship between leaf production and daily temperature would easily be lost due to water availability effects. Clearly, water is the ultimate limiting resource for growth during summer/autumn, to which the effect of temperature is secondary.

As daily temperature fluctuates seasonally in a fairly predictable pattern, the extent of growth within the summer growing season and to a lesser degree the length of the season (mentioned earlier) is largely limited by availability of water. Although the use of precipitation to predict phenology has often been regarded as misleading (Kummerow 1983), Fritts (1976) believes that in areas where extreme drought forms a part of the annual seasonal cycle it is a highly useful predictor of growth variation. The latter holds true for the growth of *X. preissii* where a rainfall variable was included in each regression model, and for the banksia woodland grasstrees rainfall was by far the strongest variable correlated with leaf production. Water sources present during summer include saturated and non-saturated soil storage and unpredictable summer rainfall. However, not all are available to *X. preissii* over this period. Grasstrees growing in both habitats rely on winter/spring rainfall to replenish soil water stores prior to summer drought. Poor leaf production following the unseasonally dry spring of 2000 implies that recharge of soil moisture by winter precipitation was insufficient to guarantee a normal flush of growth during early summer. *X. preissii* is a medium-rooted species, with its roots reaching to the clay subsoil (1–5 m) in the jarrah forest habitat (Crombie *et al.* 1988), and to a depth of at least 6 m on the Swan Coastal Plain. The related species *X. australis* also has an extensive root system reaching 3 m in heath vegetation, providing

this species with a competitive advantage over smaller monocotyledons during periods of drought (Specht 1981).

In the absence of rain during summer, *X. preissii* still exhibits some leaf elongation and low rates of leaf production. Although this implies root contact with soil moisture, it also testifies to a rooting depth unable to access permanent groundwater resources. At least on the Swan Coastal Plain, the great depth to groundwater in banksia woodland near the YC site (minimum depth = 12 m and maximum depth = 50 m; Water and Rivers Commission) precludes *X. preissii* from using this resource. Grasstrees are extremely responsive to summer precipitation, a trait observed for a number of other species in mediterranean Australia (Specht 1957b, Lamont 1976, Crombie 1992). But as seen in this study the effect of this source of water varies on the habitat. Differences in the seasonal pattern of water availability and utilisation are a consequence of the broadly contrasting substrates with different moisture relations on which they grow, that in turn reflects the two distinctly different vegetation types dominating their habitats (Beard 1983).

Annual patterns of predawn and midday water potential were consistent with data collected for *X. preissii* from the jarrah forest by other authors (Crombie *et al.* 1988, Crombie 1992), yet values during summer drought were comparatively less negative than other medium-rooted species (Dodd *et al.* 1984). With the progressive drying of surface soil during summer, Ψ_{PDX} for *X. preissii* from both habitats reaches a seasonal low in mid-autumn, following minimum Ψ_{MDX} associated with mid-summer and the high diurnal maximum temperatures. However, at no point were the grasstrees water-stressed, evidenced by Ψ_{PDX} maintained well above the Ψ_{TLP}. More specifically, this result indicates that sufficient water was available for cell expansion and hence growth (Meidner & Sheriff 1976), as leaf growth measurements show. Consistent with these findings, Crombie *et al.* (1988) showed that *X. preissii* Ψ_{PDX} was maintained higher than for deep-rooted jarrah trees during summer, and explained that very low stomatal conductance and low rates of transpiration for *X. preissii* were responsible (Crombie *et al.* 1988, Crombie 1992). In light of this explanation, continuous summer-autumn growth and the maintenance of high Ψ_{PDX} must be met with a fine balance between water loss and CO_2 uptake. During periods of drought, plants manage stomatal conductance to optimise the relationship between water loss and CO_2 uptake, which

describes water use efficiency (Mooney 1986). While midday stomatal conductance for *X. preissii* is low during mid summer (0.05 cm s^{-1}, Crombie 1992), possibly restricting growth, it is likely that late afternoon and especially morning rates would be significantly greater. In mediterranean habits radiation levels after dawn are sufficiently high and evaporative demand sufficiently low to improve plant water use efficiency, and consequently stimulate growth (Savé *et al*. 2000).

The greater responsiveness of *X. preissii* to summer rainfall on the coastal plain most clearly illustrates the dichotomy of the soil properties between habitats. Water is available to plants growing on the coastal sands at low tension because of the low colloid content (Benecke & van der Ploeg 1981, Bettenay 1984). Effectively, plants growing on sandy soils have an advantage over those growing on the heavier soils of the Darling Range, as the sandy soils require less moisture to bring the soil back from an air-dry condition into the available moisture range (Bettenay 1984). A minimum of approximately 18 mm of rain is sufficient to rehydrate the soil in the banksia woodland to a level available to *X. preissii* and penetrates deeply enough to reach active roots. In contrast jarrah forest soils would require more than 18 mm of rainfall to achieve an equivalent moisture level, before *X. preissii* could take up water to support new growth. This obvious difference between grasstrees from the two habitats rules out the possibility of water uptake via the leaves following rain (Rundel 1982), which was suggested by the adhesive nature of wet grasstree leaves, retaining a film of water in close contact with much of the crown. Water-repellency of the surface sand during summer, caused by the accumulation of hydrophobic substances (eg. plant waxes), would influence this minimum rainfall quantity necessary for growth (McArthur 1991, Roper 1999).

The results from the simulated rainfall experiment (59.2 mm) indicate that water uptake by grasstree roots occurred in the upper 33 cm of soil, suggesting that water absorption following 18 mm of rain would also be within a third of a meter of the surface. However, it is likely that rain interception by the foliage (Specht 1957a), or the natural umbrella effect of a large skirt of air-dry thatch, may contribute to determining the effective penetration of rainfall immediately below a grasstree. By the end of summer the proximal end of the primary roots within this zone (upper 33 cm) are predominately of a decorticated nature. The dying of the cortex is considered to occur in the roots'

second year (Pate & Canny 1999), yet observations from the current study indicate that this process may begin earlier, during the first summer drought period. Irreversible structural adjustments of roots in response to decreasing soil water availability were demonstrated for an evergreen sclerophyllous tree species growing in the Mediterranean basin (Psaras and Rhizopoulou, unpublished, in Rhizopoulou & Davies 1991). Although the structural changes reported in this study involved the development of periderm (cork), the resulting low water permeability of these roots would be similar for the decorticated roots of *X. preissii*, which are tightly bound by a strong sheath of lignified fibres (Pate & Canny 1999). During the wet-season these decorticated roots produce fine (< 1.5 mm), short-lived feeding roots (Pate & Canny 1999), which are unlikely to survive to provide water uptake following sporadic summer rainfall. Therefore, it may be speculated that the distal end of at least the new seasons' lateral roots must persist in an intact form through summer and autumn, providing *X. preissii* with sufficient permeable root surface to take advantage of water available during temporary soil rehydration, following unseasonal rain. Because of the severe dryness characteristic of the upper soil layer by late summer (McArthur & Bettenay 1974), it is likely that these shallow, laterally orientated roots would rely on the redistribution (hydraulic lift) of water from deeper profiles via vertical roots to sustain them in a condition capable of water uptake immediately following rain. Hydraulic lift has been demonstrated to recharge the lateral roots of *Banksia prionotes* from the Swan Coastal Plain with ground water from the tap root during the night when demand by the shoot for water would be at a minimum (Pate *et al.* 1998, Burgess *et al.* 2000). Also, the sandy root-sheath observed to encrust grasstree roots probably offers protection of these roots against desiccation and heat stress during the summer (Dodd *et al.* 1984). The information gathered from studying the rapid water status and subsequent leaf production response of *X. preissii* growing on the Swan Coastal Plain to artificial watering in autumn, clearly demonstrates that this species remains in an active state throughout seasonal drought.

Leaf shedding can occur under extreme prolonged water deficit. However, with the exception of *Phyllanthus calycinus* (Bell & Stephens 1984), regular summer deciduousness cannot be supported by the relatively infertile Australian soils (Monks 1966). Instead, physiological control of leaf water relations is a more commonly adopted strategy employed by Australian flora for surviving drought. Annual leaf production by *X. preissii* was balanced by leaf death (dead leaves are retained) during 1999/2000, so

that no substantial net increase or decrease in leaf area would have occurred. A similar trend has been shown for heath vegetation in mediterranean South Australia (Specht 1957b). *X. preissii* leaf death occurred with great certainty during mid-summer each year, corresponding with the period of fastest leaf production. Both the timing of leaf death and the age of leaves at death were consistent with *X. reflexa* (Specht *et al.* 1981) and consistent with observations made of other Australian species (Specht & Rayson 1957a, Gill 1964 from Specht & Brouwer 1975, Bell & Stephens 1984). The period during which leaf death occurs remains relatively constant from year to year, yet the rate of leaf death for *X. preissii* increased in response to drier summer conditions. As leaf production in *X. preissii* responds negatively to dry summer conditions the annual ratio of leaf death to leaf production, and therefore crown size, has the potential to change annually dependent on summer growing conditions. It is then reasonable to assume that the effect of either a poor or favourable summer may carry a legacy, in relation to crown size (photosynthetic capacity), into the following year. In equatorial latitudes palms show no season of active leaf fall or replacement, thus the crown is maintained continuously (Staff & Waterhouse 1981). Unlike tropical palms, grasstrees experience a season of drought, where bulk death of living foliage occurs. This drought induced leaf death is consistent with results shown for a drought tolerant species of coastal sage (up to 2 m) in California (Kolb & Davis 1994). However, leaf drop in the sage species was closely coupled with extreme water potentials and embolism of xylem tissue (Kolb & Davis 1994), which is definitely not the case in *X. preissii*. In this grasstree leaf death appears to be triggered by reduced water availability and represents a phenological event that acts to reduce water stress imposed by high transpiration rates from a crown of maximum annual size.

2.4.4 Verification of the grasstree ageing and fire history technique

One of the primary objectives of the work described in this chapter was to identify seasonality of grasstree growth and to correlate annual leaf production with the number of leafbases within each pair of seasonal bands, in an attempt to validate the ageing technique described by Ward *et al.* (2001). Here, verification of the technique has been limited to these specific objectives, and a more comprehensive discussion can be found in Chapter 6. The indistinguishable difference between the number of leaves produced annually by *X. preissii* and the number of leafbases contained in one pair of seasonal growth bands (one annual growth increment) confirms this technique. The findings

presented here also support the assumption that the alternating cream and brown bands on the stem reflect seasonal changes in growth (Ward *et al.* 2001). The ageing component of the technique relies on a pattern of continuous annual growth characterised by two distinct seasonal phases, established by my study.

If *X. preissii* had not revealed these growth characteristics it should not have been possible to differentiate between adjacent annual growth increments as required by the ageing technique. This interpretation is further supported by a comparison of grasstree growth responses from the two habitats studied. The weaker seasonal pattern of leaf production by grasstrees in the banksia woodland explains the difficulty experienced in identifying annual growth increments compared with the jarrah forest grasstrees that exhibit a more distinct seasonal growth rhythm.

CHAPTER 3

Effect of fire on grasstree growth

3.1 Introduction

While many features of *X. preissii* describe a plant struggling to endure the destructive force of fire, other characteristics highlight a dependency. Dead leaves are retained on the stem of *X. preissii* and accumulate below the living foliage as a grey skirt. The highly flammable skirt is easily ignited during even the mildest of fires, creating an intense, short-lived inferno (Fig. 3.1a,b), which can generate high (+600 ˚C), localised temperatures (Roy Wittkuhn, PhD student, Curtin University, pers. comm.). This feature of grasstrees, consistent with the hypothesis of Mutch (1970) of evolved flammability, ensures that the plant is burnt, yet minimises the period of exposure to lethal temperatures (Gill 1981b, Recher & Christensen 1981).

Fig. 3.1 (a) the dry, dead skirt of *X. preissii* (1.5 m tall) burns rapidly with high intensity during dry conditions. The speed at which the crown is consumed by fire prevents the prolonged exposure of the terminal meristem to temperatures that may result in its death, (b) leaving the grasstree (in this case three all under 0.8 m tall) with a tuft of charred green leaves.

Grasstrees rely on the protection of the single apical meristem to resprout after fire. Staff (1970) has documented non-fatal injury of the shoot apex, as the result of decapitation and significant caudex damage, of *X. australis* and likens it to the reactions observed for other arborescent monocotyledons that contain secondary thickening meristems (Tomlinson & Zimmermann 1969). During this study a single individual of *X. preissii* on the roadside was opportunistically photographed while sprouting from the stem, after losing its terminal apex probably during road maintenance (Fig. 3.2). Even still, recovery from fire damage (by producing multiple adventitious shoots) as the result

Fig. 3.2 A chest height specimen of *X. preissii* resprouting new leaves from below the plant's apex. The apical meristem was lost through mechanical damage, probably associated with disturbance during road maintenance. This grasstree was located beside a gravel road in the Darling Range.

of either total apical scorch or stem damage, caused by high fuel loads resting against the stem and creating localised hot-spots, was not observed in this study and appears to be relatively uncommon. In areas of high moisture in the Warby Range state park, Victoria, 3–10% of the resident grasstrees (*X. australis*) developed epicormic shoots after their stem fractured or their shoot apices died following fire (Curtis 1998). Mortality of individuals of *X. australis* as the result of prescribed burning has led to viewing fire as a negative pressure on this species (Curtis 1998). Yet for grasstrees in the

tuart forest south of Perth, Western Australia, long periods of fire exclusion have led to rot, termite damage and the accumulation of massive thatch loads, which can weaken the stem of taller plants and create lethal temperatures during the next inevitable wildfire (Ward 2000, Ward & Lamont 2000).

Fire alters the physical and chemical properties of a plant's environment (for a summary see Jeffrey 1987), and exposure of aboveground parts present causes injury, but also stimulation. For resprouting species, successful recovery in the postfire environment is equally as important as survival of the fire itself. Grasstrees are renowned for their ability to rapidly resprout following fire, despite the loss of almost all their foliage (Fig. 3.1b). Specifically, *X. preissii* is the first species to demonstrate new growth following fire (Baird 1977). Rates of crown growth after fire by resprouting species are poorly studied, or tend only to suggest that it is initially rapid (for a brief summary see Rundel 1981), offering a competitive advantage over nonsprouting species (Vlok & Yeaton 2000). Several studies of fire succession have documented the time taken for a particular plant community or species to achieve pre-fire biomass, cover or even general appearance (Specht *et al.* 1958, Baird 1977, Bowen & Pate 1993). However, few provide detail of how long the stimulatory effect of fire lasts, and whether it is sustained long enough for a species to reproduce while still under its influence.

Specht and Rayson (1957a) recorded a steady increase in leaf dry weight of *X. australis* over 11 months following fire in heathlands of South Australia. In Queensland leaves are produced at a rate of two per day for *Xanthorrhoea johnsonii* in the first 200 to 240 days after fire; beyond this the effect of fire greatly diminishes with this rate decreasing to half by 600 days postfire (Bülow-Olsen *et al.* 1982). This time frame for a fire effect is comparable to that of woody shrub species in the Mediterranean region of southern France (Trabaud & De Chanterac 1985). Growth ring analysis of the canopy species, *Eucalyptus marginata* (jarrah), which co-occurs with *X. preissii* in the mediterranean climate of southwestern Australia, indicated that after an initial period of no growth immediately following fire, this species experiences a surge of growth lasting for up to 4–5 years (Wallace 1965). Although this finding is broadly accepted as the typical response of jarrah to highly intense fires (Bell *et al.* 1989), work by Abbott and Loneragan (1983) did not paint such a clear picture of the effect of fire on this species. In their study, the effect of frequent low intensity burns on the growth rate of jarrah was

demonstrated to be indifferent, while the effect of a high intensity fire reduced growth in one instance and increased it in another.

Identifying the specific environmental factors responsible for the characteristic spurt of growth after fire has been central to a substantial amount of work and speculation (for review on the subject see Naveh 1974, Fritts 1976, Christensen *et al.* 1981). Much of this work has contributed to our knowledge of the effect of fire on the extent of fire stimulated growth and growth phenology. Nutritional enrichment of the soil from ash created by the incineration of plant material, which in the case of grasstrees includes the persistent dead leaves, is a likely growth stimulant, and is a factor ranked high in importance for rapid postfire growth (Gardner 1957, Biswell 1974, Christensen & Muller 1975, Rundel 1981, Pate & Dell 1984, Jeffrey 1987).

While canopy thinning (Stoneman & Schofield 1989) and overstorey removal (Stoneman *et al.* 1995) in the jarrah forest, and burning of heath vegetation (Specht 1957b) conserves soil moisture by reducing water loss through transpiration, a negative effect of fire on soil moisture has also been reported. Christensen & Muller (1975) explain how the removal of the shrub cover, which increases the insolation incident upon the blackened soil surface, results in increased evaporation from the upper soil layers in burned chaparral. It is possible that both conflicting phenomena may operate within the one habitat, but to different extents. Removal of the overstorey in the jarrah forest, analogous to the action of fire, leads to increased soil temperatures favouring growth of *E. marginata* seedlings (Stoneman *et al.* 1995). In addition to promoting growth, it is reasonable to assume that fire may also play a role in the timing of growth. Specht and Specht (1999) provide a comparable explanation to that of Stoneman *et al.* (1995), discussing how premature warming of understorey meristems initiates early shoot and floral growth of the ground stratum in the Dark Island heath community. In a review, Daubenmire (1968) describes how elevated soil temperatures as the result of low plant cover after fire promotes precocious leaf growth of various grass species, and that this effect is reduced or absent in successive years, as the vegetation cover increases.

Most environments have a period during the year when the probability of fire is at a peak. For the mediterranean climate in southwestern Australia this coincides with the dry, hot summer (Gill 1975, Walker 1981). Aside from natural ignition and unauthorised

lighting of fires due to vandalism or negligence, the majority of fires occur outside this period during the less hazardous months of the year via prescribed burning, when the risk of uncontrolled wildfire is reduced. Particularly in the northern limits of the Southwest, the chance of human-caused fire can extend appreciably beyond the dry summer and autumn (Walker 1981). Season of fire could have a significant effect on some processes: facilitating erosion by exposing the soil close to the wet season, influencing postfire habitat temperatures, disrupting reproduction, and promoting or inhibiting postfire resprouting and flowering (Cremer 1973, Biswell 1974, Gill 1981a, Negrón-Ortiz & Gorchov 2000, Clendenin & Ross 2001). For grassland communities, the influence of the timing of fire is almost as important as the fire itself (Daubenmire 1968).

For species that flower in response to fire, the rate of crown regeneration may be critical to later reproductive success. It is well documented that grasstrees flower in response to fire (Specht *et al.* 1958, Gill & Ingwersen 1976, Bülow-Olsen *et al.* 1982, Taylor *et al.* 1998), *X. preissii* is no exception, commonly flowering in the first spring after fire (Baird 1977, Lamont & Downes 1979, Baird 1984, Lamont *et al.* 2000). Consequently, the length of the regenerative period will depend upon the timing, or season, of fire in relation to the start of the reproductive season. If postfire crown size has important implications for the reproductive success of *X. preissii*, then season of fire should be an essential consideration for managing this species. Crown recovery following discrete fire seasons is assessed in this chapter, and the significance of the crown to reproduction is explored later in Chapter 4.

Rapid regrowth of shoots following fire involves an inherent cost in terms of carbon (Miyanishi & Kellman 1986, Malanson & Trabaud 1988, Bowen & Pate 1993). Thus the allocation of carbohydrate resources to well-protected energy reserves is essential for resprouting species. The common occurrence of starch as an energy reserve for resprouting species on the Swan Coastal Plain has been well established (Pate & Dixon 1981, Pate & Dixon 1982, Pate *et al.* 1990, Bowen & Pate 1991), and the magnitude of this reserve is regarded as a primary limitation of a plant's capacity to produce new shoot biomass (Woods *et al.* 1959, Bowen & Pate 1993). These reserves are most commonly accumulated in the root system, often forming a lignotuber, a swollen region

of the lower stem or upper root system, usually within 10 cm of the soil surface (Dodd *et al*. 1984).

The only record of carbohydrates stored as an energy reserve in grasstrees of the genus *Xanthorrrhoea* is of starch grains stored in the desmium tissue of the stem (Staff & Waterhouse 1981). The desmium is analogous to the cambium of many woody dicotyledons. However, rather than producing externally located phloem, the desmium gives rise to internal, discrete vascular bundles and secondary parenchymatous derivatives to the outer edge, called anomalous secondary growth (Staff & Waterhouse 1981). The desmium is a narrow, white, soft band of cells encompassing the living core of the stem of arborescent grasstrees, constituting the secondary meristem unique to this and other monocotyledonous genera (Tomlinson & Zimmermann 1969, Staff & Waterhouse 1981). Curiously, this tissue is continuous with the primary meristematic tissue at the apex (Staff & Waterhouse 1981).

Like other species in temperate regions, grasstree carbon reserves are likely to fluctuate seasonally (Bamber & Humphreys 1965, Huddle & Pallardy 1999, Cruz & Moreno 2001). These reserves fluctuate in accordance with the physiological status of the plant. Northern Hemisphere examples indicate that starch reserves peak during late autumn, are reduced in winter to supply basal metabolic needs, and then fall again during leafout in spring (Bonicel *et al*. 1987, Malanson & Trabaud 1988, Kozlowski & Pallardy 1997). If recovery of *X. preissii* after fire depends on adequate desmium starch reserves, then seasonal disparity of those reserves would imply a further consequence of the season of fire to the species resprouting success. Autumn fire has been reported to have a detrimental effect on resprouting species because of the prior depletion of accumulated starch reserves over the growing season (Bradstock & Myerscough 1988).

While Chapter 2 established an appreciation of grasstree growth phenology, this chapter draws on this information for comparison, uncovering the effect of burning on the growth of *X. preissii*. An understanding is developed of this species' relationship with fire, including the close relationship of reproduction with the occurrence of fire (more of which is discussed in Chapter 4). Grasstrees from within the jarrah forest and banksia woodland were examined, and the significance of season of fire is considered as a likely

factor influencing the recovery of this species. The main hypotheses investigated during this study were:

1) Resprouting patterns of *X. preissii* in the jarrah forest and banksia woodland reflect the phenological differences associated with the two environments (Chapter 2). While jarrah forest grasstree leaf production was predicted to adhere more closely to an even, seasonally undulating pattern, banksia woodland grasstrees were expected to demonstrate a higher degree of variability particularly during the summer/autumn season when these grasstrees were shown to respond readily to substantial rainfall (Chapter 2).
2) Vigorous resprouting by *X. preissii* decreases with time since fire, yet persists at least until the reproductive season in the first spring following fire.
3) Water availability is a primary factor contributing to the positive effect of fire on leaf growth, so that grasstrees in burnt sites experience increased predawn water potentials, most noticeably soon after fire.
4) Timing of fire influences the resprouting success of *X. preissii*. As *X. preissii* experienced lowest leaf production and elongation during winter and highest during spring/summer (Chapter 2), it was predicted that resprouting success would be partially dependent on the coincidence of fire with this annual cycle. Specifically, it was expected that *X. preissii* would resprout more vigorously after spring fire than after autumn fire.
5) Starch stored in the desmium is required by *X. preissii* to support initial postfire recovery, and is depleted with time.

3.2 Materials and methods

3.2.1 Study sites

Sites used for this work were located in jarrah forest on the Darling Range (MC, MSB98 and MAB00) and banksia woodland on the Swan Coastal Plain (YC, YSB98, YAB99 and YAB00). Within each of these two habitats several locations with similar vegetation structure were used. Four sites were selected, two scheduled for a spring prescribed burn (MSB98 and YSB98) and two for an autumn prescribed burn (MAB00 and YAB99; YAB00 was added later), conducted by the Department of Conservation and Land Management (CALM). The two unburnt sites (MC and YC; Fig. 1.2a,b), used for work described in Chapter 2, remained unburnt for comparison. Because of unfavourable autumn burning conditions during 1999 and 2000 on the Darling Range, several individual grasstrees were spot burnt (29 May 2000), including a 1–2 m radius of surrounding vegetation (Fig. 3.3), to create the jarrah forest autumn burnt site (MAB00).

Fig. 3.3 An intense spot burn of a target grasstree and surrounding vegetation from the autumn-burnt jarrah forest site (MAB00, 29 May 2000), and below, the same area two hours after burning, with the target grasstree (centre) pruned in preparation for leaf growth monitoring (see Section 3.2.2.2). Note the target grasstree on the far left of the upper plate is 96 cm tall from ground to stem apex.

All sites within each habitat type were located within 12 km of each other. Details of each site are described in Table 1.1, and habitat locations are mapped in Fig. 1.3. Temperature and rainfall data for these two distinct habitats are presented in Section 2.2.1 (Fig. 2.1).

3.2.2 Direct measurements of leaf production, elongation and death

3.2.2.1 The relationship between stem circumference and leaf production

Sites used for leaf production monitoring were selected from a limited number with suitable fire history and vegetation structure, and individual grasstrees of workable heights were chosen from within these (as described in section 2.2.2). During this work it became apparent that grasstree stem circumference was positively correlated with leaf production (r^2= 0.785; Fig. 3.4). A one-way ANOVA was conducted to compare the stem circumference of each plant (0.4 m from the ground) at each of the six sites. Stem circumferences of grasstrees at the spring-burnt, banksia woodland site YSB98 were

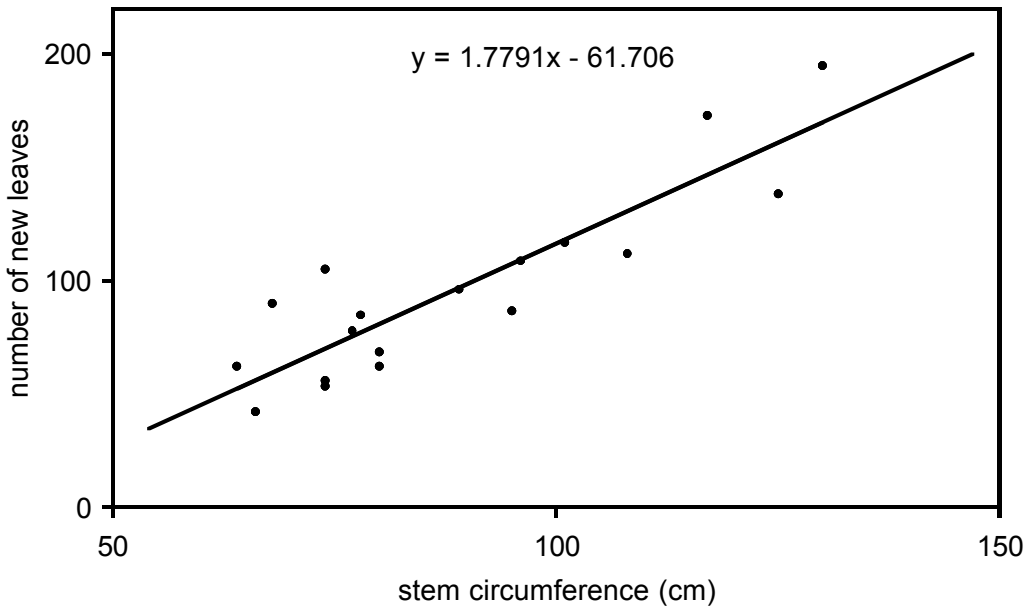

Fig. 3.4 Relationship between *X. preissii* stem circumference and leaf production rate. For 18 unburnt grasstrees, stem circumference was measured 40 cm above the ground and the number of leaves produced over 83 days (April to July 2000) was counted. Grasstrees were from three sites: two from banksia woodland and one from jarrah forest.

significantly larger than at the other sites (Table 3.1). So that site comparisons of grasstree leaf production could be easily made, the higher rate of leaf production at this

site, suggested by larger stem circumferences, was standardised. Six unburnt grasstrees from an area immediately adjacent to the YSB98 site, size-matched with the six YSB98 spring-burnt grasstrees, were monitored for leaf production (as described in Section 2.2.2) over 104 days. Leaf production from this period for the burnt and unburnt YSB98 grasstrees and the unburnt YC grasstrees was analysed using a one-way ANOVA and $\log_{10}$ transformed to meet the assumption of homogeneity of variance. A suitable adjustment was then made to standardise the leaf production results of the spring-burnt YSB98 grasstrees on the basis of these results. See results for details.

Table 3.1 Mean ± standard error (SE) for stem circumferences of *X. preissii* used at each site for leaf production monitoring (n = 6). Lower-case letters indicate results of Tukey's analysis for stem circumferences, concluded from ANOVA to be significantly different across sites.

Site	Stem circumference (m)	Tukey's
MC (jarrah forest)	0.75 ± 0.03	a
MSB98 (jarrah forest)	0.67 ± 0.03	a
MAB00 (jarrah forest)	0.70 ± 0.03	a
YC (banksia woodland)	0.80 ± 0.04	a
YSB98 (banksia woodland)	1.11 ± 0.04	b
YAB99 (banksia woodland)	0.81 ± 0.03	a
One-way ANOVA	$P < 0.0001$	

3.2.2.2 Direct measurement of leaf production and elongation

At each burnt site, monitoring of leaf production and leaf progression to the next size category (≅ elongation) for *X. preissii* commenced within 12 days of fire. Leaf growth and death monitoring was carried out according to the method described in Section 2.2.2. Burnt grasstrees required that the crown remaining after fire be trimmed using secateurs so that each plant started with a similar amount of foliage. This was determined by measuring the circumference of the crown of leaves when gathered up above the apex. After each plant was assessed, the foliage was reduced to match the smallest circumference measured. Tagging of leaves on burnt grasstrees followed the same procedure as unburnt, except that tagging of intermediate and mature leaves was not required immediately, as these leaf categories had been removed by fire or trimming. All sites were visited consistent with the monitoring of unburnt grasstrees. Rates of leaf

production and leaf progression were calculated and contrasted against the equivalent results for the unburnt grasstrees. Also, spring-burnt grasstree leaf production was compared with leaf production predicted using the second regression model from Section 2.2.2 (Methods) and 2.3.1.1 (Results), and the corresponding climatic variables (maximum single rainfall event for each growth period, and day length). The purpose of this last comparison was to apply the modelling from Chapter 2 in an attempt to further assess its suitability for predicting grasstree leaf production.

Leaf age at death was calculated for the single cohort of young leaves present at the time of fire, for both spring-burnt and unburnt grasstrees in jarrah forest and banksia woodland. The first leaves tagged for each of the six grasstrees at each site (Section 2.2.2) comprised the cohort of young leaves that was monitored. For each grasstree, the time taken for the first leaf from the original group of young leaves to die was recorded.

3.2.3 Root growth

New root sampling was undertaken at the second autumn-burnt banksia woodland site (YAB00), on the same dates as the root work conducted at the unburnt banksia woodland site (YC). Soil was excavated from around the base of grasstrees size-matched with those from YC, and new roots identified and sampled according to the method described in Section 2.2.4. Processing of collected roots was also carried out at the same time as those collected from the unburnt site and handled in the same manner. A minimum of six grasstrees was excavated on each visit to the YAB00 site. Nine days postfire (2 June 2000), a brief preliminary investigation, independent of the unburnt site, was conducted to determine the prevalence of new roots immediately after fire and as a check for the start of the root-growing season. This simply involved excavating grasstrees until one was found with new roots.

As for the root work described in Chapter 2, the percentage of grasstrees with new roots was calculated, and a binomial test was used to determine if the occurrence of new roots was dependent on season (sample sizes were too small to meet the assumptions of the preferred chi-squared contingency table analysis). From the roots collected the proportion of new roots in each root tip condition category (see Fig. 2.3, in Chapter 2) was determined. Also, seasonal differences and the interaction between burnt and unburnt sites in the estimated total new root biomass, the number of roots per grasstree

and root size (g/cm) were assessed using a two-way ANOVA. Log_{10} transformation was used to meet the assumptions of this analysis.

3.2.4 Postfire xylem water potentials

Xylem water potential (Ψ_X) was measured predawn (Ψ_{PDX}) and midday (Ψ_{MDX}) for grasstrees at each burnt site used for leaf monitoring, concurrent with measurements made at the unburnt sites (see Chapter 2 for detailed results of the unburnt sites). Both spring-burnt sites were visited from late November 1998 to April 2000. The banksia woodland autumn burnt site (YAB99) was visited from May 1999 until April 2000, while the jarrah forest autumn burnt site (MAB00, burnt 13 months after YAB99) was visited from late July 2000 to June 2001. The distal 15 cm of one leaf was collected from a random sample of six grasstrees, from which Ψ_X was measured (Scholander *et al.* 1965) using a pressure chamber (PMS Instrument Company, Oregon, USA).

Water potential at turgor loss point (Ψ_{TLP}) was determined for a sample of three to six grasstrees at each burnt site using the method described in Section 2.2.5.1, and compared with the results of unburnt plants from Chapter 2. Ψ_{TLP} was measured in autumn for grasstrees from MSB98 (3 April 2000), YSB98 and YAB99 (30 March 2000), and in summer for grasstrees from MAB00 (8 December 2000).

3.2.5 Stored energy

A preliminary investigation was carried out to eliminate the possibility that starch was stored elsewhere within the plant, other than in the desmium (Staff & Waterhouse 1981), and to investigate the broad extent of the desmium starch reserve. Freshly excised tissues from different components of grasstrees from the spring (YSB98) and autumn burnt sites (YAB00) from the banksia woodland were stained with 2% iodine (KI and I_2 were dissolved in water at a ratio of 1:1 by weight). Extra tissue from the roots and desmium was taken back to the laboratory for closer examination using light microscopy. Samples of caudex tissue from 3 grasstrees were preserved in glutaraldehyde and stored in 50% ethanol solution (by volume) for later sectioning and more detailed examination of the desmium tissue. This preserved tissue was later embedded in Paraplast tissue embedding medium (paraffin wax and plastic polymers; Oxford Labware, USA), sectioned (12 μm) using a rotary sledge microtome (820

Spencer microtome, American Optical Corporation, USA), and then affixed to slides. Each slide was treated with either 2% iodine (1 KI : 1 I_2 by weight in water) to locate starch, or periodic acid-Schiff's reagent for staining insoluble carbohydrate and counterstained with amido black for highlighting protein bodies (O'Brien & McCully 1981, Phillips & Schneider 1981). The stained sections were then covered with a glass cover slip held with a polyvinyl-alcohol mounting medium (Omar *et al.* 1978). Slides were examined using a light photomicroscope (Olympus, VANOX-S AHBS-513) and photographs taken at magnifications 8–100×, using Kodak 100ASA film.

3.2.5.1 Use of stored energy following fire

Autumn burnt sites in the jarrah forest (MAB00) and banksia woodland (YAB00) were chosen for this work. However, due to adverse burning conditions in the jarrah forest only a small number of grasstrees was individually burnt, not enough to support this work. Grasstrees from YC and YAB00 were sampled 22 days prior to fire and then on four occasions following fire. Destructive sampling was avoided by drilling 38–mm-diameter holes into the side of each grasstree 2/3 of the way up the stem, through the protective leafbases. Drilling ceased once the white parenchymatous secondary cortex could be seen (or just prior with careful judgement). A cork corer was then used to systematically remove pieces of the underlying tissue (secondary cortex, desmium and fibrous secondary vascular bundles) as 12–mm-diameter plugs. Excised tissue was placed in labelled plastic tubes and stored within an insulated bag cooled with ice until reopened at the lab.

A transverse section of the desmium was cut by hand for each sample. The sections were stained with 2% iodine solution (1 KI : 1 I_2 by weight in water) before washing and mounting in a water/glycerol medium. Using a dissecting microscope (Nikon, Japan) with an ocular graticule, the width of the visible band of cells containing starch granules was measured at 20× and 30× magnifications, and then the mean of these two values calculated. A measure of the colour intensity of the starch-containing band was recorded using one of four preselected categories from colour charts (Royal Horticultural Society, London, England). Each colour score was converted to a numerical value by scanning the four categories and reading off their grey scale values using the public domain NIH Image 1.62f software program (developed at the U.S. National Institutes of Health and

available on the Internet at http://rsb.info.nih.gov/nih-image/). An index of available starch was calculated as;

Starch index = band width (mm) × colour intensity (grey scale).

A number of hand-sections were photographed using a dissecting microscope (Olympus SC35 camera mounted on an Olympus SZH10 research stereo).

3.2.6 Statistical analyses

All statistical data analysis was performed using SPSS 10.0 software program (SPSS Inc., Chicago, U.S.A.). Analysis type used is indicated in the related text, and $\alpha < 0.05$ was used to determine significance. Examination of residual stem-and-leaf plots, as well as the calculation of the Shapiro-Wilk's statistic was used to test for normality. Levene's test was employed to test for homogeneity of variances. To ensure that the assumption of sphericity had not been violated for repeated-measures analysis, Mauchly's test was used. Based on the outcome of this data screening, square root or $\log_{10}$ transformations were used as required. All transformed data are presented as untransformed means, and all error terms provided are standard error.

3.3 Results

3.3.1 Direct measurement of leaf production, elongation and death

3.3.1.1 Relationship between stem circumference and leaf production: standardising leaf production to compensate for large stem circumference

Leaf production of the YC grasstrees was considerably lower than the leaf production for both burnt and unburnt grasstrees at the YSB98 site (Table 3.2). The Tukey's multiple comparison tests indicated that these differences were significant (YC versus YSB98 burnt, $P = 0.005$ and YC versus YSB98 unburnt, $P = 0.033$). In the absence of a correlation between stem circumference and leaf production it would be expected that leaf production for the larger unburnt grasstrees at YSB98 and the smaller unburnt grasstrees at YC (Table 3.1) be the same. However, as this was not the case (Fig. 3.4), to allow comparisons of YSB98 grasstree leaf production with grasstrees from other sites, leaf production (Lprod) for the YSB98 spring-burnt grasstrees was standardised (Std) as follows:

Std Lprod YSB98 burnt = Lprod YSB98 burnt × (Lprod YC/Lprod YSB98 unburnt).

All leaf production rates given for the YSB98 site are standardised results.

Table 3.2 Mean (± SE) number of leaves produced over 104 days (6 April 2000 to 19 July 2000) for three groups ($n = 6$) of *X. preissii*, burnt or unburnt and with large or small stem circumference. Lower-case letters indicate results of Tukey's analysis for the number of leaves produced shown from ANOVA to be significantly different across groups (a, higher value).

Grasstree attributes	Number of leaves produced	Tukey's
Unburnt, large stem (YSB98)	170.17 ± 4.24	a
Spring-burnt, large stem (YSB98)	190.67 ± 3.33	a
Unburnt, small stem (YC)	113.00 ± 1.30	b
One-way ANOVA	$P = 0.005$	

3.3.1.2 The effect of fire on leaf production

Vigorous resprouting of *X. preissii* was obvious, regardless of habitat or season of fire (Fig. 3.5a,b), clearly seen as leaf production above that of the controls (Fig. 3.5a,b) and that predicted for unburnt grasstrees by the second leaf production model described in Chapter 2 (Fig. 3.5c,d). The response to burning was immediate: all burnt plants produced considerably more leaves in the first growth period measured than those unburnt. Spring fire triggered the greatest initial response (Fig. 3.6), with equivalent leaf production rates in both habitats (6.0 ± 0.7 leaves/day for the jarrah forest and 6.1 ± 0.7 leaves/day for the banksia woodland). However, the maintenance of these elevated rates varied between habitats, with the spring-burnt, jarrah forest plants first reaching the unburnt rate within 32 weeks, contrasting with the 12 weeks it took the spring-burnt banksia woodland plants to attain the same level. The point where leaf production of the spring-burnt grasstrees and that predicted by modelling converged (Fig. 3.5c,d) provided a comparable means of estimating the duration of the initial period of elevated leaf production. Grasstrees burnt in the jarrah forest produced three times as many leaves (mean = 709.7 ± 50.2) as unburnt plants (mean = 251.3 ± 47.1) over this period, and grasstrees burnt in the banksia woodland produced over four times as many leaves (mean = 332.8 ± 14.4) as those unburnt (mean = 77.2 ± 10.2). Leaf production was significantly higher than unburnt plants throughout the entire growing season ($P < 0.0001$, repeated-measures), despite extended dry periods. In contrast, although a more gradual increase in leaf production at the start of the growing season was apparent for spring-burnt grasstrees in the jarrah forest compared with unburnt plants, there was no difference in overall leaf production for the 1999/2000 growing season. Mid-autumn 2000, 19 months after the banksia woodland spring burn, the leaf initiation rate for burnt plants fell to unburnt levels, where they remained through winter and into the 2000/01 growing season, marking the end of the fire effect. These observations of spring-burnt versus unburnt grasstree leaf production were also evident from the comparison of burnt grasstree leaf production and the modelled data (Fig. 3.5c,d). The number of leaves produced by spring-burnt grasstrees in both habitats in the first year following fire was not significantly different ($P = 0.505$, *t*-test): 804.1 ± 64.5 for the jarrah forest and 749.3 ± 46.0 for the banksia woodland. These annual totals represented 2.2 (jarrah forest) and 1.8 (banksia woodland) times the leaf production of the unburnt grasstrees.

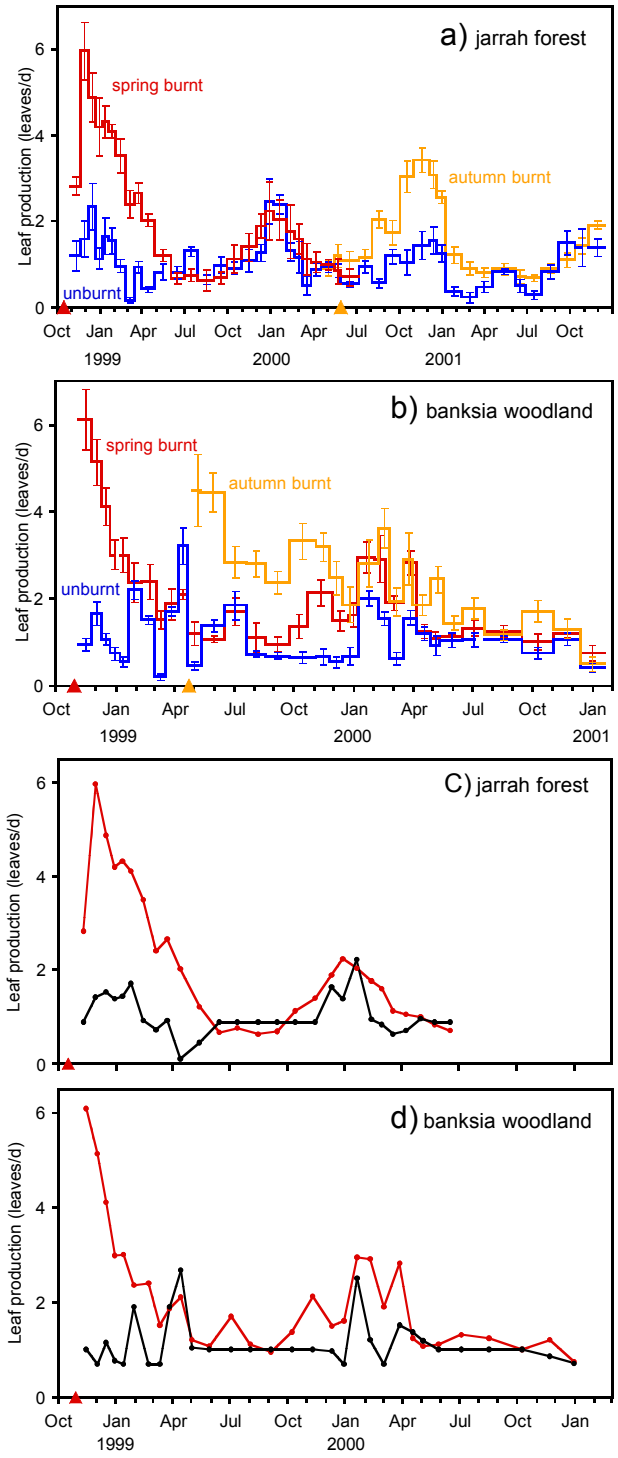

Fig. 3.5 Rate of leaf production for *X. preissii* (1998–2001) in (a and c) jarrah forest and (b and d) banksia woodland, comparing (a) and (b) two burnt sites (spring-burnt (—) and autumn-burnt (—)) and a third unburnt (—). (c) and (d) show the spring-burnt (—) site with predicted (—) spring-burnt leaf production based on the second regression model from Chapter 2 (see Section 2.2.2). Actual data are mean leaf production of six plants for each measurement period (± SE in a and b), and predicted leaf production values were calculated from the mean of each climatic variable over the same period (summer), or designated as a constant (winter). The timing of each fire is indicated by an appropriately coloured triangle on the x-axis.

Fig. 3.6 The spring-burnt (October) banksia woodland site (YSB98) 5 days after a relatively patchy burn (top), with a single grasstree pruned in preparation for leaf growth monitoring (on the right). The same site 19 weeks (March) later after the initial postfire flush of leaf growth (bottom), shown by the amount of new leaf growth on the grasstree to the right.

Of all the burnt sites, the least significant initial response was recorded for jarrah forest grasstrees burnt in autumn (Fig 3.5a). Despite being spot burnt, the consequence of late winter rain and medium fuel loads meant that the grasstrees were subjected to quite high intensity fires, with flame height reaching above 12 m in one instance (Fig. 3.3). The timing of the fire responsible for this effect was by far the latest in the year, three days from the calendar start of winter and equivalent to over one month following the autumn fire in the banksia woodland (Fig 3.5b). Mean daily air temperature for the month following autumn fire was 3 ˚C cooler in the jarrah forest than in the banksia woodland. No immediate fire response was observed for the autumn-burnt jarrah forest grasstrees,

but when rapid resprouting did commence it was in early August. Maximum leaf production (3.4 ± 0.3 leaves/d) was reached in late spring/early summer, a considerably earlier response than when the autumn-burnt banksia woodland grasstrees entered their first growing season since fire. *X. preissii* in the banksia woodland rapidly attained maximum leaf production immediately after fire (4.5 ± 0.8 leaves/d), before dropping during winter.

After commencement of the postfire leaf flush, grasstrees in both habitats maintained an elevated rate of leaf production beyond that of the unburnt grasstrees. Coinciding with the start of winter, 18 months after fire, leaf production rates for *X. preissii* at the autumn-burnt, banksia woodland site dropped to the unburnt grasstree level, and a further two months saw the permanent absence of a fire effect. Eleven months after fire, autumn-burnt grasstrees in the jarrah forest exhibited a significant convergence in leaf production towards the unburnt grasstree level. After one year following fire, the mean total number of leaves produced for the autumn-burnt jarrah forest grasstrees was 593.3 ± 31.4, a little over half of that produced by the autumn-burnt, banksia woodland grasstrees after the same period (1041.8 ± 154.4 leaves). These annual leaf totals represented 1.9 times the number of leaves produced by the unburnt jarrah forest grasstrees over the same period and 2.4 times the number of leaves produced by the unburnt banksia woodland grasstrees.

3.3.1.3 The effect of fire on leaf elongation and death

In comparing leaf progression (elongation) rates of burnt and unburnt grasstrees, the effect of spring and autumn fire in both habitats was in much the same way as their effect on leaf production (Fig. 3.7a–d). In the jarrah forest, leaf progression indicated a greater effect of spring than autumn fire across both leaf categories. This was consistent with leaf elongation of the intermediate leaf category for the banksia woodland grasstrees, but not for the young leaf category. Comparing the two habitats (Fig. 3.7a,b), young leaf elongation for the banksia woodland grasstrees showed substantially more fluctuation, alternating between high and low progression rates, relative to unburnt plants. The dynamic nature of the banksia woodland grasstrees was closely synchronised with leaf production for spring-burnt grasstrees (Fig 3.5b) and to a lesser extent for autumn-burnt grasstrees, and persisted for 8.5 months and 8.0 months respectively after

fire. Following this period, young leaf progression reflected a steadier, slowly declining trend. Young leaf-elongation for spring and autumn-burnt jarrah forest grasstrees was less dynamic, exhibiting a pattern consistent in timing and relative magnitude to leaf production. The only exception to this description was the significant increase in the leaf progression rate of spring burnt grasstrees during July 1999 (Fig. 3.5a). Young leaf-progression of autumn-burnt grasstrees from the jarrah forest shared key features observed for leaf production. The postponed start and retarded postfire flush (relative to the response by banksia woodland grasstrees) was apparent in young leaf elongation.

The positive effect of spring fires on grasstree intermediate leaf progression was similar in both habitats (Fig 3.7c,d), and was most obvious during favourable growing periods. For example, during the peak of the growing season (early summer) and immediately post-drought, spring-burnt grasstrees experienced significantly elevated intermediate leaf growth. However, during annual drought, late-summer/early-autumn, intermediate leaf progression was not much greater than that observed for the unburnt plants. All spring-burnt grasstrees experienced an early growth response during the first spring following fire and did not recover from the unburnt levels following the autumn low response. The effect of autumn fire on intermediate leaf progression was far less pronounced, for both jarrah forest and banksia woodland grasstrees. A small increase was measured for both habitats, which only become distinguishable from the unburnt grasstrees in the jarrah forest after eight months following fire. This period was noticeably shorter (five months) for the banksia woodland autumn-burnt grasstrees to show a response of equal magnitude.

Observations of leaf death among burnt grasstrees were hindered by the extensive time taken from initiation to death (Fig. 3.8). This time frame was reflected in the percentage of leaves dying each year on burnt grasstrees. Leaf death occurred during mid-summer for all grasstrees regardless of burning treatment (Fig. 3.7e,f). During the last monitored summer period (2001) the percentage of leaf death for burnt plants remained less than that for unburnt grasstrees. Leaf age at time of death was calculated for both burnt and unburnt grasstrees from both habitats (Fig. 3.8). Because plants could only be monitored for a maximum of three summers following fire and as some of the burnt grasstrees flowered during this period, sample sizes varied and were generally low. Despite mean leaf age at death being greater for unburnt grasstrees than burnt grasstrees, as the

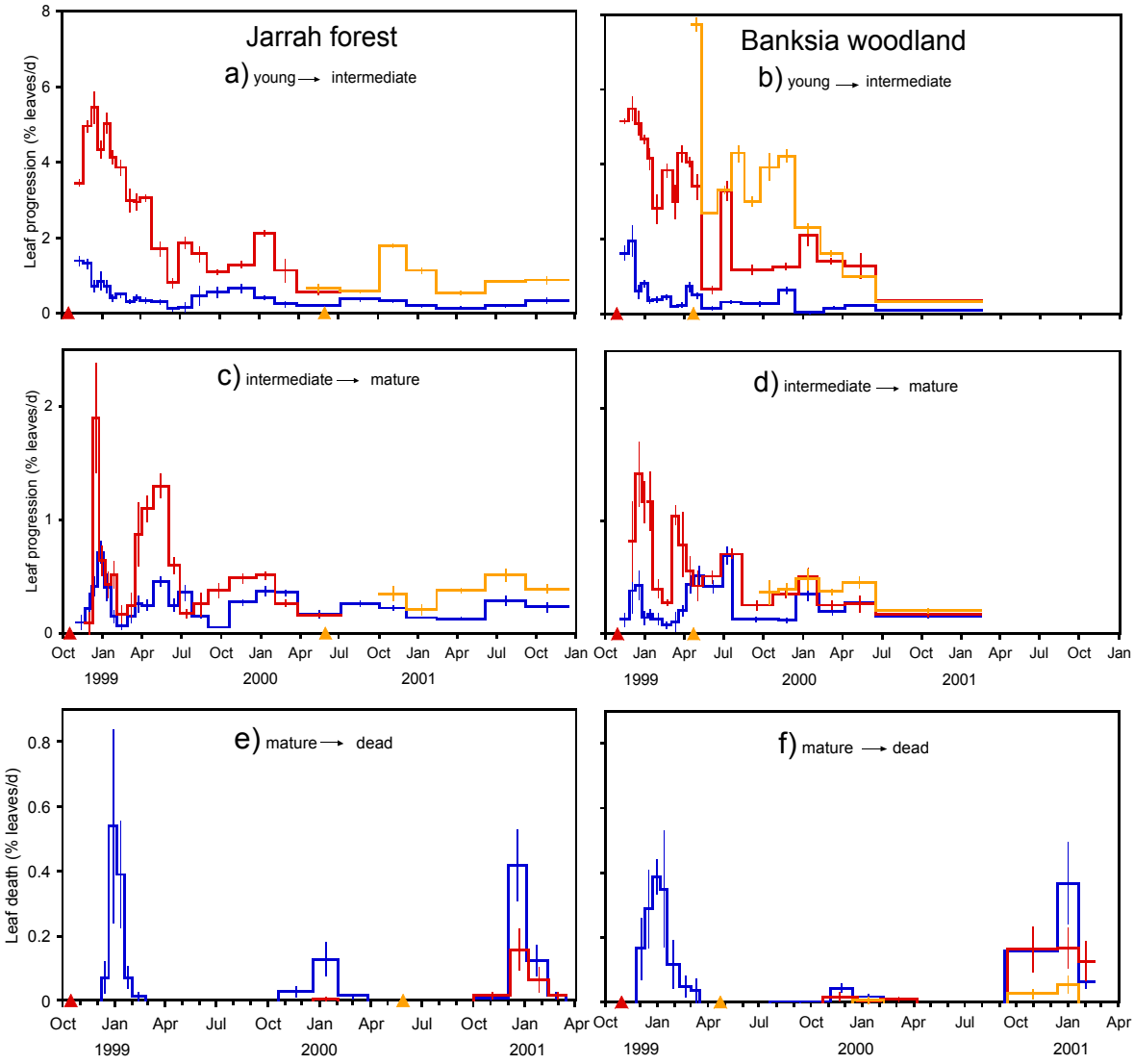

Fig. 3.7 Leaf elongation (a–d) and death (e and f) of *X. preissii* growing in jarrah forest and banksia woodland from 1998 to 2001. In each habitat, leaf elongation and leaf death were monitored at two burnt sites (spring-burnt (—) and autumn-burnt (—)), and one unburnt site (—). Leaf elongation is expressed as two phases of leaf development: (a) and (b) the percentage of young leaves that progressed to the intermediate leaf category per day, and (c) and (d) the percentage of the intermediate leaves that progressed to the mature leaf category per day. (e) and (f) leaf death is the percentage of mature leaves that die per day. Data are the mean of six plants for each measurement period (horizontal line) ± SE, and the timing of each fire is indicated by an appropriate coloured triangle on the x-axis.

standard errors suggest, this is unlikely to be very different. Larger standard errors for the burnt plants were a reflection of the strongly seasonal death of foliage. Burnt

grasstrees experienced 'tagged' leaf death over two consecutive years, while all unburnt grasstrees experienced 'tagged' leaf death only over the second of the two summers, resulting in the lower standard errors. This disparity between samples prevented the assumption of homogeneity of variances to be met, thus preventing statistical support.

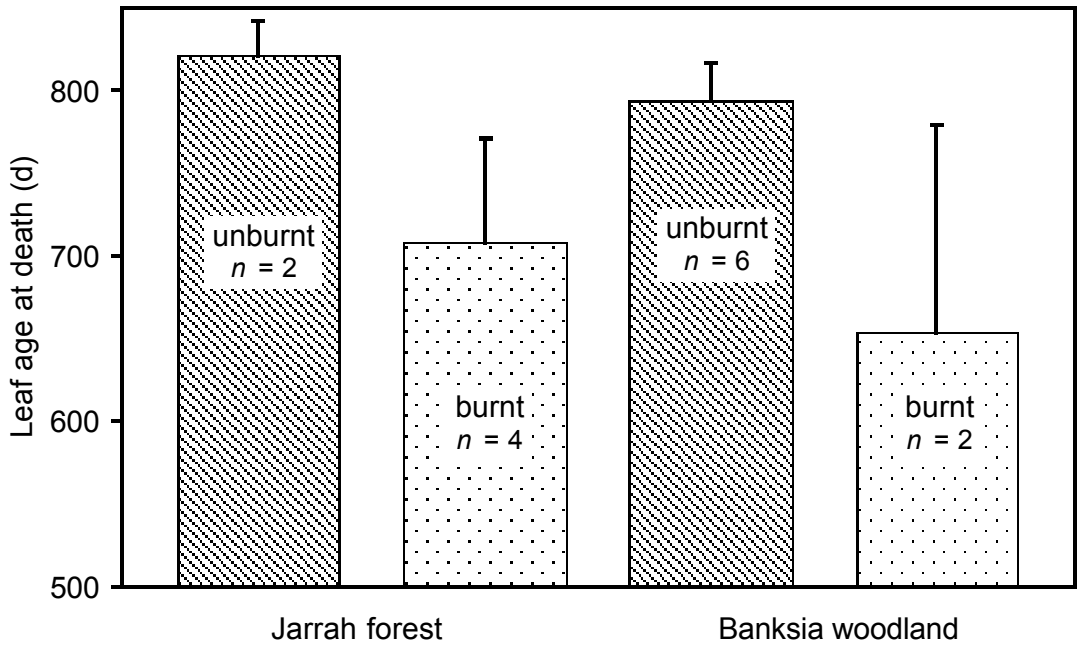

Fig. 3.8 Age of *X. preissii* leaves at death from one unburnt (hatched) and one spring-burnt (stippled) site, in jarrah forest and banksia woodland. Data are means ± SE.

3.3.2 Root growth

Data from immediately following the autumn fire revealed few new roots as expected, as the timing was prior to the onset of winter rain, which was suggested earlier to trigger root growth (see Discussion in Chapter 2). Of seven grasstrees sampled, only one had new roots (14%). Surprisingly, the probability of finding a grasstree with new roots (p) was not significantly different from 50% ($P = 0.0625$, binomial test), thus rejecting the alternative hypothesis, $p < 0.5$, but this result was marginal. The probability of finding new roots during the first year following an autumn-burn was demonstrated not to be dependent on season. The highest percentage of grasstrees with new roots was in spring, with 83%. This was the same percentage as that for the unburnt site during winter, and not greatly different from the percentage in spring from the same site (100%). However, as a likely consequence of sample size, statistically the chance of finding no new roots during spring at the burnt site was equally likely ($P = 0.109$, Table 3.3). Similarly, the

results of the binomial test did not support the prediction that fewer than 50% of autumn-burnt grasstrees possessed new roots during summer, despite only 33% of those sampled having new roots.

Table 3.3 Number of grasstrees growing at an autumn-burnt site (YAB00) in banksia woodland with new roots during each season. The number of grasstrees with new roots (n_2) is expressed as a percentage of the total number of grasstrees excavated in each season (n_1). For each season, the probability (p) that a grasstree will bear new roots was analysed using a one-tailed binomial test. As it was predicted that dry conditions in summer and autumn would not be conducive to new root production, H_O: $p = 0.5$ (50%; no seasonal effect) was used for all tests, while H_A: $p > 0.5$ was used for winter and spring, and H_A: $p < 0.5$ was used for summer and autumn. An outcome statement indicating the probability of finding new roots (p) is given in parentheses, based on the results of each test.

Site	Season	n_1	n_2	% of grasstrees with new roots	Binomial test (P)
YAB00	winter	11	7	63	0.274 ($p = 0.5$)
	spring	6	5	83	0.109 ($p = 0.5$)
	summer	6	2	33	0.109 ($p = 0.5$)
	autumn	10	5	50	0.623 ($p = 0.5$)

Two-way ANOVA revealed a significant effect of season on all three new root characteristics (Table 3.4). Total estimated new root biomass was at least 4–7 times greater during winter and spring than during summer and autumn, the significance of which was highlighted by the results of the Tukey's multiple comparison test ($P < 0.0001$, Table 3.4). The number of roots per grasstree demonstrated a similar pattern, with *X. preissii* bearing significantly fewer new roots in summer and autumn than the two wetter seasons ($P < 0.0001$, Table 3.4). *X. preissii* root size was significantly different between winter and spring ($P = 0.046$, Table 3.4); during spring mean individual root mass per unit length was greater than during winter. Root characteristics were not significantly different between the burnt and unburnt sites, and no interaction between the effect of site and season was evident (Table 3.4).

In keeping with the root characteristics data (Table 3.4), root tip condition suggested a seasonal effect, but no obvious effect of burning (Fig.3.9). The proportion of grasstree

new roots with growing tips was highest in winter and spring, and lowest in summer (no growing root tips found), followed by autumn. New root dormancy was equivalent through winter and spring, prior to more than doubling in summer: all new roots at the autumn burnt site were dormant. In autumn, at the burnt site, a small portion of excavated new roots were growing, but the majority were still dormant. Root rot was more prolific during the wetter months, peaking during spring.

Table 3.4 A summary of *X. preissii* new root attributes during each season (August 2000, November 2000, February 2001 and May 2001) for an unburnt site (YC) and a site burnt in autumn (YAB00). Root mass, number and size are the mean (± SE) of the number of plants with new roots (*n*). Results of a two-way ANOVA are provided to compare differences between all seasons and sites (burnt/unburnt). Similar letters indicate seasons whose means were not significantly different (Tukey's multiple comparison test, $P < 0.05$).

Site	Season	*n*	Total new root mass/grasstree (g)	No. new roots /grasstree	Root size (g/cm)
YC	winter	5	11.7 ± 1.4 a	14 ± 2 a	0.05 ± 0.01 a
	spring	5	42.3 ± 9.7 a	24 ± 8 a	0.13 ± 0.04 b
	summer	4	1.6 ± 0.5 b	4 ± 1 b	0.07 ± 0.00 ab
	autumn	1	0.4 b	2 b	0.07 ab
YAB00	winter	7	20.5 ± 4.4 a	24 ± 5 a	0.07 ± 0.01 a
	spring	5	39.8 ± 10.4 a	32 ± 9 a	0.09 ± 0.01 b
	summer	2	0.7 ± 0.2 b	2 ± 0 b	0.07 ± 0.01 ab
	autumn	5	4.7 ± 2.3 b	5 ± 1 b	0.13 ± 0.03 ab
Two-way ANOVA (*P*)					
Season			< 0.0001	< 0.0001	0.046
Site			0.306	0.783	0.568
Season × site			0.313	0.469	0.242

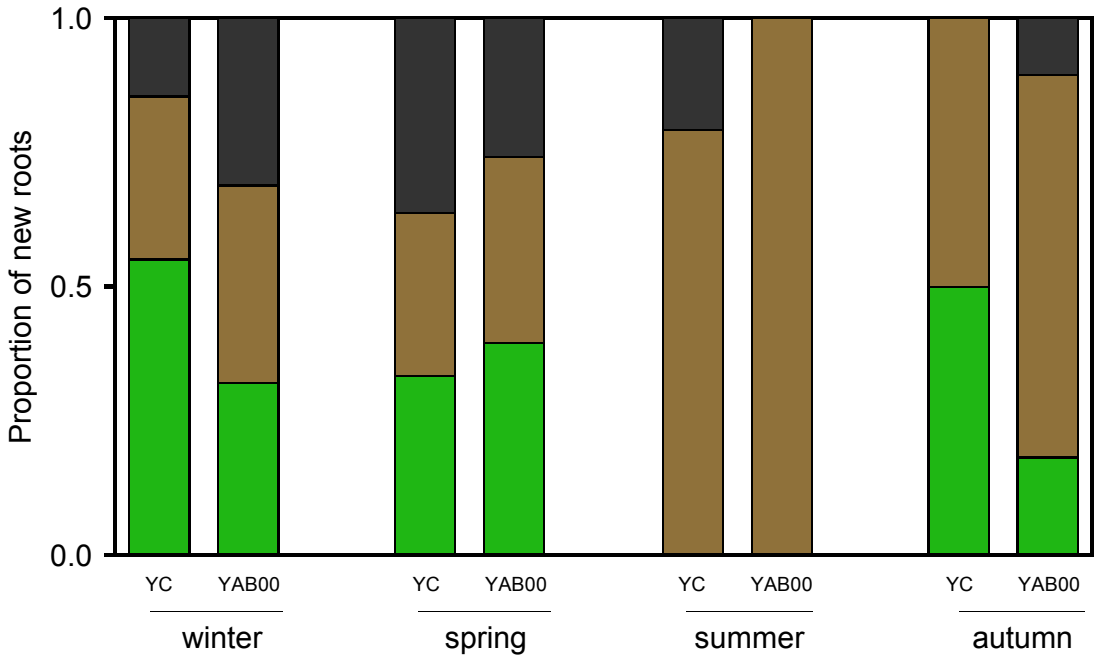

Fig. 3.9 Seasonal variation in the proportion of grasstree new roots allocated to each of three categories of root tip condition, from one unburnt (YC) and one autumn-burnt (YAB00) site in the banksia woodland. The three categories were; 1) growing (■), 2) dormant (■), and 3) rotten (■) (see Fig. 2.3 in Chapter 2 for details of these conditions). Roots were excavated during each season from 2000 to 2001 (August, November, February and May). Proportions are means (for *n* see Table 3.4), except for the YC autumn data that are based on root sampling from a single grasstree.

3.3.3 Postfire water status

In both habitats, burnt grasstree Ψ_{PDX} and Ψ_{MDX} closely followed the unburnt seasonal trends (Fig. 3.10a–d). The only deviation of burnt grasstree Ψ_{PDX} from the unburnt pattern occurred during autumn 1999, coinciding with 30 mm of unseasonal rain. During the dry season burnt and unburnt jarrah forest grasstree water potential at turgor-loss point (Ψ_{TLP}), was not significantly different ($P = 0.830$ for comparison of MC and MSB98 grasstrees during autumn and $P = 0.172$ for comparison of MC and MAB00 grasstrees during summer, two-tailed *t*-test). Because of this result the respective data were pooled. Ψ_{PDX} remained considerably higher than the combined Ψ_{TLP} (mean Ψ_{TLP} was −2.08 ± 0.16 MPa ($n = 6$) during summer and −1.78 ± 0.14 MPa ($n = 9$) during autumn). Similarly, no significant difference between burnt (spring and autumn) and unburnt banksia woodland Ψ_{TLP} during autumn ($P = 0.066$, one-way ANOVA) suggested

that the results could be pooled. Ψ_{PDX} for these grasstrees also never fell below this combined mean Ψ_{TLP} (mean of −1.99 ± 0.14 MPa, n = 13).

During the first summer following the spring burn, burnt grasstrees from both habitats had significantly higher Ψ_{MDX} (less negative) than unburnt grasstrees (P = 0.014 for the jarrah forest and P = 0.006 for the banksia woodland, one-tailed t-test, Fig. 3.10c,d). Similarly, during the first spring following fire autumn-burnt banksia woodland (4.5 months after the fire) Ψ_{MDX} became significantly higher than the unburnt (P = 0.012, one-tailed t-test). By the following April Ψ_{MDX} for these autumn-burnt grasstrees was equivalent to the unburnt grasstree Ψ_{MDX} (Fig. 3.10d). Autumn-burnt jarrah forest grasstree Ψ_{MDX} did not exhibit an equivalent deviation from the unburnt grasstree Ψ_{MDX} (Fig. 3.10c).

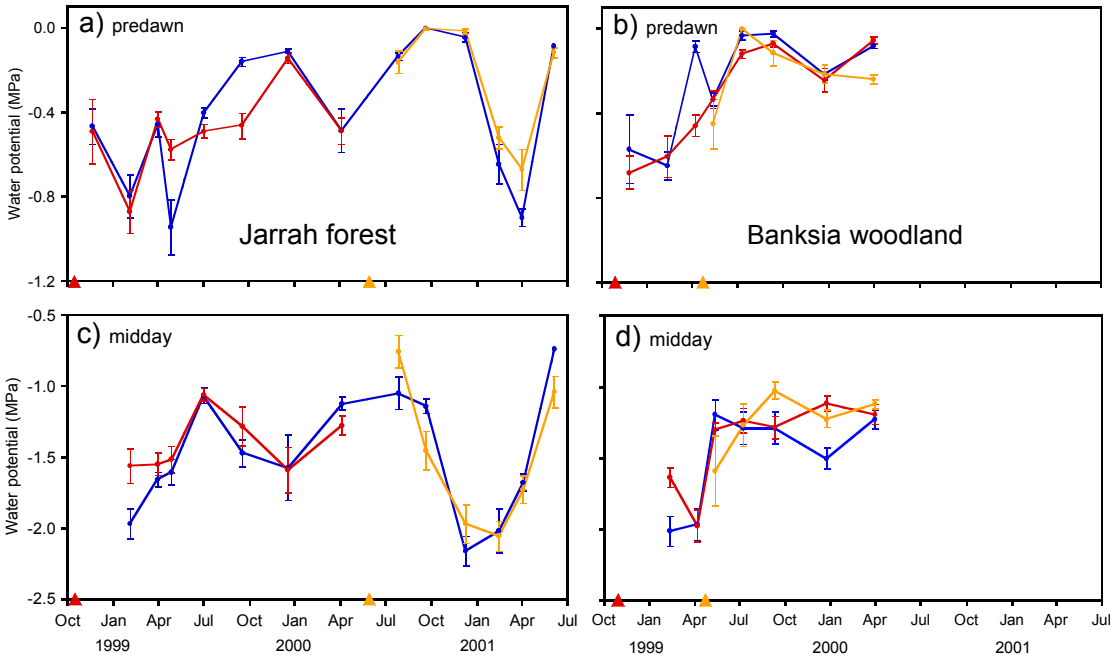

Fig. 3.10 Seasonal (a) and (b) predawn and (c) and (d) midday xylem water potential for *X. preissii,* from jarrah forest and banksia woodland, between 1998 and 2001. For each habitat data are presented for two burnt sites (spring-burnt (●) and autumn-burnt (●)) and one unburnt site (●). Data are the mean of six plants ± SE, and the timing of each fire is indicated by an appropriate coloured triangle on the x-axis.

3.3.4 Stored energy and its use following fire

Staining techniques revealed the presence of starch associated with the desmium tissue. Aside from this, traces of starch were seen in some young roots (cortex intact) on the

edge of the cortex adjacent to the stele; however, this was not consistent with all young roots and was absent from the older roots (no living cortex). The desmium was visible as a band of tissue containing groups of four or five rectangular cells, separating the inner secondary vascular tissue from the outer parenchymatous derivatives (Fig. 3.11a). Staining with Schiff's reagent and counter-staining with amido black indicated the presence of starch grains and protein bodies within the desmium (Fig. 3.11b).

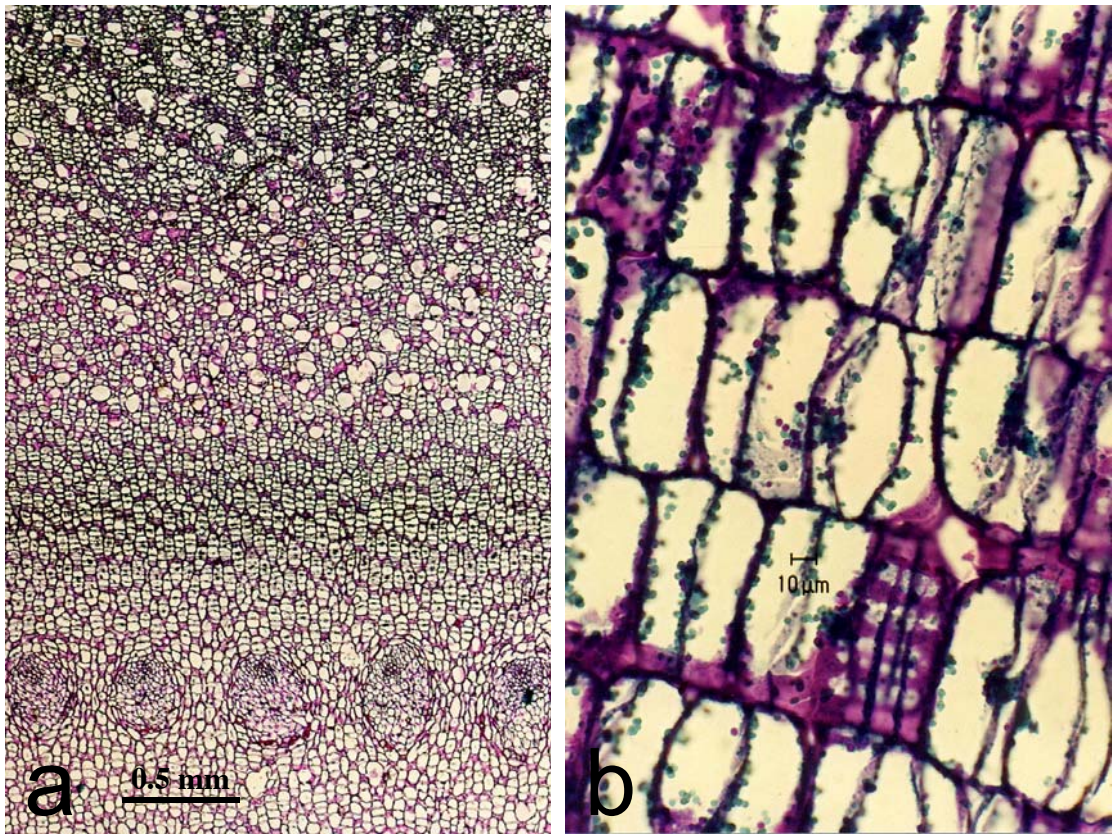

Fig. 3.11 Histology of the desmium and surrounding tissue of *X. preissii*. Material was embedded in wax and 12–µm sections treated with periodic acid-Schiff's reagent to stain insoluble starch, and with amido black to stain protein bodies. (a) at 40× magnification the desmium can be seen as a darkened band of tissue separating the inner secondary vascular bundles (below) from the parenchymatous derivatives (above) on the outer edge of the cortex. (b) starch grains (pink) and protein bodies (black) are present within the rectangular cells of the desmium.

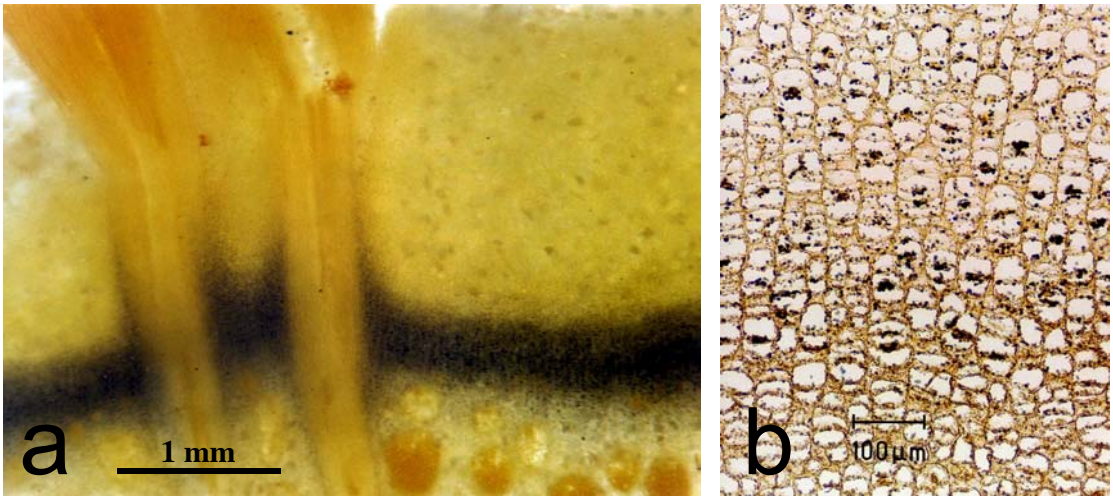

Fig. 3.12 *X. preissii* desmium tissue sections treated with 2% iodine (1 KI : 1 I_2 by weight in water). (a) fresh 560 µm section of the outer cortex displaying the dark starch band of the desmium. Two remnant leaf traces, once connecting the growing leaves with the inner vascular tissues, seen at the bottom of the photograph, disrupt the starch band. (b) wax embedded 12 µm section clearly highlighting the dark staining starch grains, concentrated in the desmium, responsible for the black band seen in (a).

Protein bodies were in greater abundance than starch, and were also densely distributed in the outermost cells of the parenchymatous tissue. Compared with fresh sections, it seemed that during the preparation of wax-embedded sections some starch was lost (Fig. 3.12).

Prior to fire the starch index for grasstrees targeted to be burnt (at YAB00), and those from the unburnt site (YC) were not significantly different ($P = 0.958$, two-tailed *t*-test). After fire, burnt grasstrees exhibited a general decrease in starch index relative to the unburnt grasstrees, most obviously during summer and autumn (Fig.3.13). The difference between the starch index for autumn-burnt and unburnt plants was significant ($P = 0.001$), with no significant difference between starch index over time after fire ($P = 0.112$) or an interaction of the two factors ($P = 0.170$, two-way ANOVA). A significant difference existed between the starch index of burnt and unburnt grasstrees in February ($P = 0.035$) but this was not present in May ($P = 0.186$, Tukey's multiple comparison test). Although ANOVA did not reveal a statistical difference in grasstree starch index over time, a seasonal trend appeared to exist. Unburnt grasstrees showed the lowest starch index around late autumn/early winter, and a peak in desmium starch during late summer.

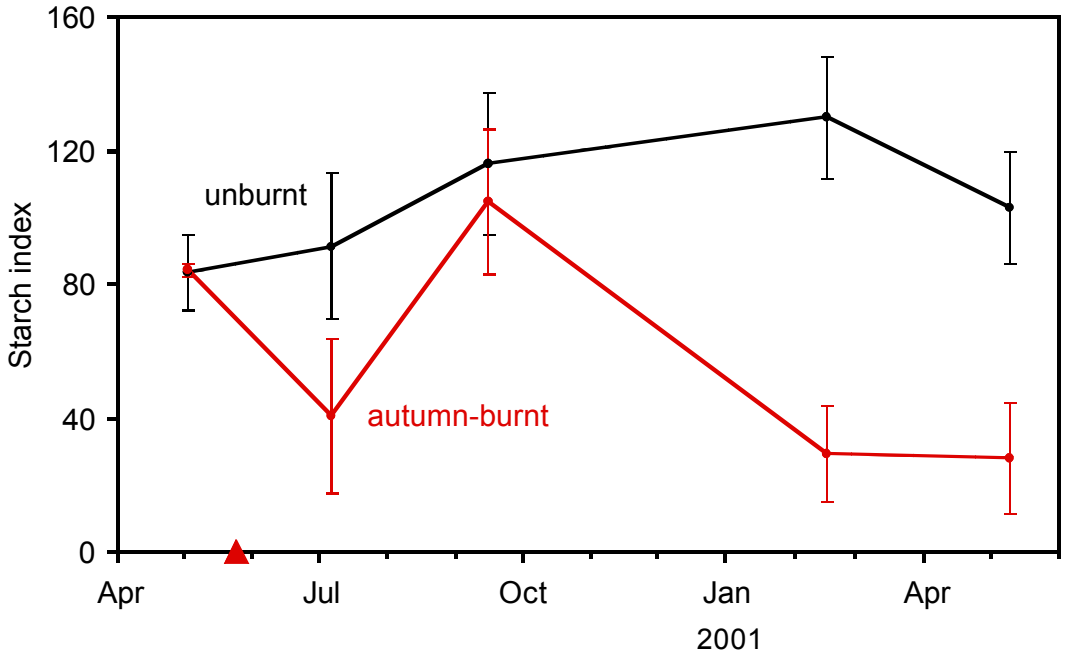

Fig. 3.13 Effect of fire on desmium starch reserves of *X. preissii* in banksia woodland. Starch index was measured for grasstrees at two sites, one unburnt and the second burnt in late autumn 2000 (24 May). One initial measurement was made prior to fire and four following. Data are the mean of three grasstrees (± SE), and the timing of the fire is indicated by a triangle on the x-axis.

3.4 Discussion

3.4.1 Rapid commencement and the extent of postfire leaf growth

Leaf growth of *X. preissii* was uninterrupted by the passage of fire, maintaining the pattern of continuous growth described earlier (Chapter 2). It was also significantly enhanced, with leaf production increasing up to six-fold shortly after fire, and was maintained at a mean rate equivalent to twice the rate observed for unburnt grasstrees during the first postfire year. In contrast, the most vigorous, deep-rooted shrub species that co-exists with *X. preissii* can take up to 2–3 weeks to resprout (Baird 1977). *Stirlingia latifolia*, a long-lived shrubby species growing on the Swan Coastal Plain, did not begin to sprout until two months after both a spring and summer burn (Bowen & Pate 1993). In keeping with *X. preissii*, Specht *et al.* (Specht *et al.* 1958) noted that regeneration of *Xanthorrhoea australis* followed almost immediately after fire, and contributed 90% of the aerial growth of the heath vegetation during the first year after fire, at Dark Island in South Australia. Unlike many resprouting species that lose their current aerial meristematic apices to fire and are forced to initiate new growth from epicormic buds originating in the cambium (Gill 1975, Bell & Koch 1980, Gill 1981a, Recher & Christensen 1981, Burrows 2002), *X. preissii* simply continues growing from the existing apical meristem that remains undamaged due to insulation from the surrounding moist, tightly packed leaves. This is consistent for other arborescent monocotyledons, some tree-ferns (eg. *Cyathea australis* and *Dicksonia Antarctica*, from the Dandenongs in eastern Australia) and cycads (Gill & Ingwersen 1976, Gill 1981a, Rundel 1981, Gill 1993), such as *Macrozamia riedlei*, that is common in the jarrah forest and banksia woodland and rapidly resprouts after fire (Baird 1977).

The rapid commencement of the postfire leaf flush of *X. preissii* would be a definite advantage for any plant re-establishing in an environment temporarily reduced in competitors yet on the brink of a very dynamic period of biomass production and recruitment. One advantage resprouting species have over other species regenerating from seeds is their well-developed root system that allows them to exploit the abundant postfire soil nutrients and available water, resulting in faster growth during the period of low competition from their counterparts, young seedlings (Biswell 1974, Keeley & Zedler 1978). Following summer fires in the banksia-eucalypt woodland of Perth's Kings Park, Baird (1977) noted a stark contrast between the rapid growth of shoots from

old root systems and the very slow growth of seedlings of the same species. However, in environments such as the jarrah forest and northern kwongan of Western Australia a high proportion of the species regenerate from rootstocks (69–70% for jarrah forest, Christensen & Kimber 1975, Bell & Koch 1980, Bell *et al.* 1989, and 66% for kwongan, Bell *et al.* 1984), which ensures a rapid return to pre-fire cover levels (Bell & Koch 1980). This implies a relatively brief 'window of opportunity' for species to benefit from low postfire competition for nutrients and soil moisture. Unquestionably, *X. preissii* is one species capable of gaining from these circumstances. From a broader lifecycle perspective, fast regeneration holds particular importance for *X. preissii*, as the passage of fire marks the start of a race against time to prepare for the upcoming reproductive season: as early as three months away for those grasstrees burnt in late autumn.

The first flush of new leaves and their subsequent elongation following spring-fire occurred so suddenly that, at least initially, new growth appeared to be triggered by the physical effect of the fire, possibly involving stimulation by the high temperatures (Negrón-Ortiz & Gorchov 2000), but more likely by the removal or death of foliage. Such an effect on crown size may reduce competition for water, nutrients and possibly starch reserves among the remaining leaves. Supporting this idea, Zammit (1988) demonstrated that the speed of resprouting of *Banksia oblongifolia* was similar after fire and clipping, even though the leading shoots of burnt plants had longer final lengths. Sustaining this heightened growth beyond the initial flush undoubtedly depends on the combined effect of the myriad of fire-related factors prevailing in the burnt habitat (Jeffrey 1987), such as the ash-bed effect. Distinguishing the individual effect of these factors on plant growth is a difficult task, and a potentially misleading one, as their combination is likely to be synergistic (see Chapter 5 for a simple experimental approach to this problem).

In keeping with the direct comparison of burnt and unburnt leaf production, the final convergence of spring-burnt grasstree leaf production and the modelled relationship predicting leaf production for unburnt grasstrees from climatic data provided a clear indication of the duration of the fire effect. In this respect, the validity of this leaf production model (first presented in Chapter 2) for predicting unburnt leaf production in jarrah forest and banksia woodland is further confirmed.

The stimulatory effect of fire on *X. preissii* did not persist for as long as some species, such as *Eucalyptus marginata* (jarrah) (Wallace 1965, Bell *et al.* 1989), but the response of this grasstree to fire was far more predictable (Abbott & Loneragan 1983). Specht and Rayson (1957a) claim a relatively constant increase in dry weight over eleven months following fire for *X. australis*. However, the last two measurements made during their study suggest a reduction in the rate of dry weight accumulation: the end of fire stimulated growth at 9–11 months? For grasstrees that did not reproduce after fire, elevated growth was maintained through the first year after fire (8–11 months in the jarrah forest) and even into the second year (up to 20 months) for coastal plain populations. This time span was very similar to the stimulatory effect of burning during the wet summer season for the subtropical cycad *Zamia pumila* (1–1.5 years, Negrón-Ortiz & Gorchov 2000), and consistent with the response for other cycad species, including *Macrozamia riedlei* (Baird 1977, Dolva & Scott 1982). This period clearly represents how long the properties of the burnt environment that were responsible for the positive effect on grasstree growth persisted.

The period following spring fire was sufficient for *X. preissii* to recover a sizeable crown and complete reproduction in banksia woodland, and was marginally shorter than the time required in jarrah forest (see Chapter 4 for details of reproduction). Assuming that the postfire interval when conditions are conducive for accelerated growth does not vary significantly between fires, it is reasonable that *X. preissii* may have adapted to exploit the burnt environment by condensing its recovery and subsequent reproduction into this timeframe. This speculation implies that a selective advantage exists for plants that reproduce soon after fire, perhaps in the form of greater seed number, size and viability. Additionally, postfire flowering by grasstrees could be driven by the benefits to seedlings from developing in an environment enriched in nutrients and reduced pressure from competitors, herbivores and diseases (Recher & Christensen 1981). This topic requires further research.

Leaf age at death indicated that for some burnt grasstrees the youngest leaves present at the time of fire senesce earlier than the equivalent leaves on unburnt grasstrees (mean difference of about 4–5 months). It is possible that this disparity was due to young leaves on burnt grasstrees experiencing detrimental effects from high fire temperatures, directly reducing their health and longevity. However, this is not supported by

observations made during a recent combustion experiment, where young leaves near the apex of *X. preissii* rarely exhibited visual damage caused by fire (Roy Wittkuhn, pers. comm.). Therefore, assuming that the fire event itself is not responsible for reduced leaf duration, another postfire effect must be involved. Leaf longevity is generally longest for plants occupying the most nutrient-deficient habitats (Mooney & Gulmon 1982). When nutrients are readily available to a plant (specifically nitrogen), Mooney (1983) explains that the faster the resultant growth rate, the faster the movement of nitrogen from old to new leaves and the shorter the life of the leaf. Since the burnt sites are undoubtedly rich in nutrients compared with the unburnt sites, it seems reasonable that the influx of nutrients lead to reduction in leaf longevity of *X. preissii* at the burnt sites. Additionally, the early leaf death two years after fire suggests that a residual fire effect may persist until this time, prolonging the effect of fire beyond that demonstrated by leaf growth.

3.4.2 Commencement of postfire growth: what happens below ground?

While leaf growth reacts markedly after fire, it is not surprising that root growth immediately following autumn fire was not observed to be very different from the seasonal patterns at the unburnt site. The belowground portion of *X. preissii* is not subjected to the same stimuli experienced above the soil surface.

A mere few centimetres of soil are sufficient to insulate subterranean plant organs from the lethal temperatures experienced at the surface (Rundel 1981). In a study of *Xanthorrhoea gracilis* (a stemless grasstree species) in jarrah forest, death of underground leaf tissue occurred during fire when soil temperatures were between 41 and 46 ˚C, beneath a recorded maximum surface temperature of 290 ˚C (Koch & Bell 1980). This lethal temperature range was experienced to a soil depth of 3 cm, below which the soil above provided adequate insulation to allow the living tissue of these plants to survive. Roots of *X. preissii* generally occur at least 7 times this depth (see Chapter 2, and Pate & Canny 1999) and as a result are unlikely to experience any significant rise in the surrounding soil temperature during fire.

Nutrient enrichment is a likely growth stimulant for the root system. However, whether the fire is prescribed (spring or autumn) or wild (most likely during summer), its timing is usually coincident with the drier period of the year when regular rainfall is

uncommon. Without significant rainfall (≥ 18 mm, see Chapter 2) soon after fire, ash-derived nutrients may not be carried down to the roots and instead, consequently lost from the environment by wind (Raison 1980).

Many mediterranean resprouting species support postfire foliar growth with a lignotuber or rootstock (Naveh 1975, Lacey 1983, James 1984, Pate *et al.* 1990, Bowen & Pate 1993). In contrast, grasstrees access carbon reserves above ground, remobilising carbohydrates from starch stored within the stem, further adding to (what undoubtedly must be) an apparent air of redundancy regarding the role of the root system in the rapid regenerative process.

It seems that if it were not for the physical connection between the subterranean and aerial portions of *X. preissii*, the opportunity would exist for the intact mature root system to remain totally isolated from any direct stimulus from the passage of fire above. Statistical evidence presented here supports this scenario, suggesting that there was no effect of the autumn fire on new root growth. But trends in the data were not always the same. It is hard to overlook the greater root number and total root biomass of the autumn-burnt grasstrees compared with those unburnt, when measured during the first winter following the fire. Three months later in spring, the burnt plants still revealed a greater mean number of new roots per grasstree. These trends could be interpreted as a stimulatory effect of the autumn fire on the start of the root-growing season, and more specifically, as the likely result of nutrient enrichment of the root zone. The small amount of rain that fell within eight days of the fire (Fig. 2.1) is speculated to have washed some of the nutrient rich ash into the surface soil. Subsequent leaching by the heavier rain that fell a further two weeks later would have aided nutrient release, carrying them down to the underlying roots. An ash leaching study (Stark 1979) suggested that these circumstances are likely to create a sudden surge in soil nutrient concentration around the roots. Leaching plant ash with 100 mL of water removed 65–68% of cations, and generally, the largest amounts of nutrients are released in the first 300 mL of water passed through a sample of ash (Stark 1979). Later, in early spring, when leaf growth commences it is plausible that the root system may experience a secondary stimulus to growth from its role in the absorption of nutrients and water, which would rapidly increase to meet the high demands of the young expanding foliage.

3.4.3 The dependence of the effect of fire on habitat and season of burn

The pattern of *X. preissii* crown regeneration was, to a degree, the product of the habitat in which the grasstrees grew and the season in which they were burnt. The dependency of resprouting on habitat was most apparent when comparing leaf production rates of grasstrees burnt in spring from the jarrah forest and banksia woodland, as these two fires shared the most similar timing (less than two weeks apart). Jarrah forest grasstree leaf production and early elongation was surprisingly unrestrained by low soil moisture during autumn, enabling these plants to maintain an elevated rate through the first growing season following fire. Having developed an understanding of the annual growth phenology of *X. preissii* (see Chapter 2 for detail), it was not surprising that leaf growth of the burnt grasstrees was continuous, as reported by Specht and Rayson (1957a) for *Xanthorrhoea australis* from a heath community. However, leaf growth of *X. preissii* did not progress independent of climatic conditions, as Specht and Rayson (1957a) also suggested, clearly decreasing with the approach of winter, as a probable result of falling daily air temperature. If one looks closely at Specht and Rayson's data, for a period of about two months over winter *X. australis* does not increase dry weight, suggesting some dependence on climate, a pattern of growth more attuned to *X. preissii* in the jarrah forest.

In contrast to the lateritic soils of the Jarrah forest, the permeable sandy soils of the banksia woodland quickly lose moisture during spring, contributing to a greater water deficit earlier in summer, particularly in the upper metre of soil (McArthur & Bettenay 1974). This distinction between the habitats, which helped explain the contrasting seasonal growth patterns of *X. preissii* from the jarrah forest and banksia woodland (see Chapter 2 for detail), can also be attributed to the difference in crown recovery between grasstrees from these two habitats over the first growing season after fire. Low soil moisture in the banksia woodland during summer and autumn quickly restricted the length of the initial flush of new leaves. Surprisingly, the pattern of early crown recovery of heath grasstrees (*X. australis*, Specht & Rayson 1957a) was more similar to that of the jarrah forest grasstrees, than it was to the grasstrees growing in the banksia woodland, despite the closer physical affinity between heath and banksia woodland. The later fire in the heath study (mid-summer) meant that the initial (strongest) surge of fire-stimulated growth, coinciding with the driest time of the year, masked the underlying

effect of low water availability, which was clearly expressed by the leaf growth of the banksia woodland grasstrees. The limiting effect of water in this habitat can also explain the greater fluctuations of both leaf production and progression in the banksia woodland grasstrees than the jarrah forest grasstrees.

Leaves progressing from the intermediate category to maturity required the greatest amount of elongation (leaves elongated from < 30 cm to > 1 m), and correspondingly this stage of leaf growth was the most susceptible to low water availability. The maintenance of turgor, crucial for leaf expansion (Meidner & Sheriff 1976), was most difficult for *X. preissii* during the combined hottest and driest period of the year, in late summer/early autumn. This period was typically characterised by an annual low level of Ψ_{PDX} for both burnt and unburnt grasstrees from both habitats and marked a synchronised decrease in leaf progression (from the intermediate to the mature leaf category) of the spring-burnt grasstrees from the two habitats during their first postfire growing season. The recovery of this rate of leaf progression to the former fire-stimulated level appeared to be the net result of lower diurnal temperatures and increasing frequency of rainfall towards winter.

The typically dry and hot summer of southwestern Australia did not restrict leaf production of burnt grasstrees to the same extent as those unburnt. The removal of foliage by fire can act to conserve soil moisture by reducing transpirational water loss (Specht 1957b, Stoneman & Schofield 1989, Stoneman *et al.* 1995). This effect could operate to enhance growing conditions at the burnt sites. Yet, no difference in Ψ_{PDX} indicated that water available to *X. preissii* was similar in the burnt and unburnt sites, independent of habitat. Part of the resolution may lie in the higher (less negative) Ψ_{MDX} of burnt grasstrees during their first postfire growing season. These higher water potentials represent a reduced demand for water by the smaller postfire crown, essentially lowering the chance of water stress despite no evident change in site water status. In this state, photosynthesis and therefore leaf growth were less likely to be reduced by the expected low stomatal conductance of *X. preissii* during summer (Crombie 1992) as a trade-off for higher water use efficiency. Water potentials from a study of two Mediterranean (Spain) shrub species used to explain the difference in growth rates of burnt and unburnt plants (Fleck *et al.* 1995) parallels with *X. preissii*. In their study, improved water relations enabled the maintenance of open stomata during

stressful conditions during summer, permitting the investment of abundant postfire nitrogen to enhance net photosynthesis. Assuming *X. preissii* experiences an increase in available nutrients after fire, a similar increase in net photosynthesis may contribute to the enhanced leaf production of burnt grasstrees relative to those unburnt.

All burnt grasstrees exhibited an earlier response to warming temperatures in spring than their unburnt counterparts, in keeping with the findings and conclusions of other researchers for native prairie grasses (Ehrenreich 1959), *E. marginata* (Stoneman *et al.* 1995) and heathland vegetation from southern Australia (Specht & Specht 1999). The conclusion drawn by Ehrenreich (1959) that precocity in new season growth was related to soil temperature could explain the findings from my study. The role of fire in effectively opening the canopy and reducing grasstree self-shading may result in an earlier rise in shoot apex temperature, stimulating an early start of new season growth. It can then be argued that the closer *X. preissii* is burnt prior to spring, the less time is available for regrowth to insulate the apex from rising temperatures at the beginning of spring. As a result these grasstrees may experience an earlier leaf production flush at the start of the growing season.

Support for the above explanation was provided by the earliest leaf flush for the growing season (3 August 2000) starting for those grasstrees burnt in late autumn (29 May 2000), closest to spring. Additionally, similar timing of the jarrah forest and banksia woodland spring burns was reflected in the minor discrepancy between the timing of the grasstree start in the second postfire growing season. It could be argued that the timing of the fire is irrelevant given that grasstrees in jarrah forest and banksia woodland exhibited different patterns of resprouting, and therefore may produce different levels of apical cover. However, the number of leaves produced by *X. preissii* in the two habitats after one-year (about coincident with the start of the growing season) were equivalent, demonstrating that they were equally successful in crown recovery. The data presented here support the idea that the early warming of the apex meristem in spring, due to the loss of cover, instigates precocious new season leaf growth. The equivalent timing of the start of the grasstree growing season in unburnt jarrah forest and banksia woodland, despite significant canopy cover differences (73% for MC (Chapter 5) and 15% for YC (unpublished)), suggests that the loss of grasstree foliage must be primarily responsible for this postfire growth phenomenon.

Generally, the seasonal variables that guide this species phenology (temperature and water availability) still underlie the fire-stimulated growth, and consequently *X. preissii* tended to be less susceptible to the negative effects of adverse growing conditions when resprouting, but growth was never totally independent of these factors. This was clearly demonstrated by the growth response of *X. preissii* from the jarrah forest when exposed to unfavourable growing conditions following the autumn fire. Timing of autumn fires is critical to maximising crown regeneration by *X. preissii*. Fire in early autumn provides burnt grasstrees with the opportunity to resprout vigorously, but the growth response to fire in late autumn was restricted by the onset of cooler winter temperatures. Chamise chaparral in California represents an extreme example of this trend and will not sprout at all until the next spring after being burnt in autumn (Biswell 1974). It is reasonable to predict that grasstrees burnt in the banksia woodland during mid-summer as the result of wildfire may resprout poorly under conditions of low water availability, although the growth response of *X. australis* (Specht & Rayson 1957a) suggests otherwise. Such delays between fire and a subsequent period when climatic conditions are favourable for growth have a negative effect on the resprouting success of *X. preissii*. This was evident in the difference between the number of leaves produced after 12 months following fire for grasstrees burnt during autumn in the two habitats (593 ± 31 for jarrah forest grasstrees and 1042 ± 154 for banksia woodland grasstrees), and also between their leaf progression characteristics. Specifically, this difference in leaf production performance can partly be attributed to the 3 ˚C warmer postfire conditions in the banksia woodland compared to the jarrah forest.

Coupling the possible variability in resprouting success associated with autumn burns and the generally lower initial rates of leaf production and elongation, regeneration by *X. preissii* seems most effective following spring-burning. Even for spring-burnt grasstrees that have the longest time to recover before the following spring flowering, leaf death rates indicate that an incomplete crown must support the inevitably high demand of carbon during reproduction. In fact, the number of leaves that die in the third summer following fire is less for burnt grasstrees than it is for unburnt grasstrees, suggesting that by this time the crown is not yet at full size (with maximum leaf turnover). Autumn-burnt grasstrees will often commence reproduction in late August, a mere 3–4 months after fire, representing the absolute minimum time period between fire and reproduction. Spring fire may therefore also favour inflorescence growth and the subsequent seed

production by *X. preissii*, although no difference in the number of fruits per spike between spring and autumn burnt jarrah forest grasstrees was shown by Lamont *et al.* (2000). However, it is possible that this study did not distinguish between those autumn burnt grasstrees that flowered in the year that they were burnt or in the following year, when the crown would be closer to complete recovery. Whether this short 3–4 month interval postfire is sufficient for *X. preissii* to recover a crown large enough to support the carbon demands of inflorescence production and reproduction is a reasonable question, which will be addressed in the following chapter. Yet, since leaf growth still shows a fire effect one-year after a spring burn, concurrent with the timing of reproduction, logic suggests that a grasstree undergoing this longer regenerative period would, at the least, not be disadvantaged and would most likely benefit.

Spring fire also offers the advantage of a longer resprouting period with reduced competition from seedlings emerging after germination in winter, contrasting with the shorter interval from autumn fire to winter germination. The reverse of this rationale has been used to support the preference of autumn burns for most effective regeneration of serotinous, nonsprouting species re-establishing after fire in the sandplains north of Perth, Western Australia (Bell *et al.* 1987). As *X. preissii* relies on dehydration of the capsule for seed release and not the passage of fire, season of fire does not offer any advantage or disadvantage in this regard.

3.4.4 The carbon cost of resprouting following fire

Staining with iodine provided an easy, clear method for demonstrating the distribution of starch in the desmium. Starch stored in the desmium tissue of the stem of *X. preissii* appears to contribute to the carbon requirements of leaf growth following fire. Autumn-burnt grasstrees in the banksia woodland exhibited a reduction in desmium starch (recorded as a significant decrease in the starch index) during the first growing season after fire, which suggests that starch was necessary for the rapid resprouting process. A similar role played by root starch reserves during resprouting after fire has been demonstrated for other species (Miyanishi & Kellman 1986), including the banksia woodland shrub, *Stirlingia latifolia* (Bowen & Pate 1993). In the absence of a full crown of photosynthetic leaves, burnt grasstrees must translocate the carbon stored as starch and direct it in a soluble form to the actively growing apex. Prior to this, it is likely that some starch may be mobilised to support the postfire winter/spring root growth

suggested by the results presented earlier. Although, statistically, starch consumption was not identified until the timing of spring leaf flush, the trend for the burnt grasstree starch index suggests a smaller decrease coincident with winter.

Unburnt grasstrees exhibited a degree of seasonal fluctuation in desmium starch reserves, consistent with findings by other researchers working on shrub species (Bowen & Pate 1993, Huddle & Pallardy 1999, Cruz & Moreno 2001). The annual trend for *X. preissii* was in agreement with that demonstrated by Bowen and Pate (1993) for the co-existing species, *Stirlingia latifolia*, suggesting that during spring and summer conditions are suitable for the accumulation of starch reserves, but prior to late autumn *X. preissii* draws most heavily on these same reserves. Cruz and Moreno (2001) concluded from the low starch reserves during spring-summer that the function of starch contained in the lignotuber of the Mediterranean species, *Erica australis,* related to the support of growth and reproduction, and a possible response to drought stress. Assuming that the fitness of a species is improved by storing starch, the function of that starch will depend upon the environmental pressures imposed on the species. For *X. preissii*, and also *Stirlingia latifolia*, the synchronisation of the annual peak in starch reserves with the most fire-prone time of the year suggests these species' capacity to store carbon has been selected primarily in response to fire. For *X. preissii* this may also explain the relatively minimal annual fluctuation in starch reserves for unburnt plants.

In my study, only starch was assessed as a nutritional requirement for resprouting by *X. preissii*. However, other studies of woody shrubs, considering an array of nutrients, have concluded that starch was the most likely nutrient to limit resprouting (Jones & Laude 1960, Miyanishi & Kellman 1986). Coincidentally, grasstrees during this study were burnt when starch reserves would have been at their lowest, yet despite some grasstrees having a starch index that approached zero, at no point was it reached. This sheds some doubt over whether the carbon stores of *X. preissii* could limit its regrowth potential, especially since other plants are known to store reserves in excess of their needs for a single resprouting episode (Van der Heyden & Stock 1995). From this it is inconceivable that low starch reserves in late autumn were solely responsible for the poor initial resprouting effort by the jarrah forest grasstrees burnt in late autumn (and not climate constraints as argued earlier). Further work is needed to rule out the possibility that low reserves may act in unison with climate to restrict the postfire performance of

X. preissii. This strengthens the case for greater efficiency of crown recovery after burning of *X. preissii* in spring. While grasstrees are not currently rare, for the future protection of some grasstree populations (eg. in the tuart forest, Ward 2000, and Warby Range State Park, Curtis 1998) consideration of prescribed burning regimes may be necessary to maximise seed production and/or to ensure vigorous post fire regeneration. Typically, timing of prescribed fire is usually determined only by pragmatic factors such as suitability of weather and availability of personnel (Mcloughlin 1998).

Protein bodies associated with the desmium have not been previously recorded for grasstrees, although Cordemoy (1893, from Tomlinson & Zimmermann 1969), who was concerned with the function of the desmium in several genera of monocotyledons, noted the occasional presence of oils in these related species. Several plant species occurring on the Swan Coastal Plain have been identified as storing various amounts of proteins as well as starch, within distinct subterranean storage organs (Pate & Dixon 1981). For these species, protein bodies and starch disappeared after germination of the storage organs, offering evidence that these substances are important food reserves for establishing new growth (Pate & Dixon 1982). The storage of protein may play a similar role in *X. preissii*, but further work on this species' use of protein is required.

CHAPTER 4

Reproduction in *X. preissii* and its associated costs

4.1 Introduction

The majority of Australia is susceptible to fire, with minor exceptions at the extremes of the continent (Gill 1975, Francis 1981). The literature on flowering responses to fire in Australian plants is not extensive (Staff 1989). However, Gill (1981a) lists the species known to exhibit this phenomenon, and more have been identified since (eg. van der Moezel *et al.* 1987). Of interest is the dominance of monocotyledons on this list, including several species in the genus *Xanthorrhoea*. Grasstrees known to flower following fire include *X. australis* (Specht *et al.* 1958), *X. johnsonii* (Bülow-Olsen *et al.* 1982), *X. fulva* (Taylor *et al.* 1998), and *X. preissii* (Meagher 1974, Lamont & Downes 1979, Ward & Lamont 2000), all of which flower far less frequently in the absence of fire (Gill & Ingwersen 1976, Baird 1977).

While attempting to identify the individual components of the fire effect responsible for triggering reproduction in grasstrees (Fig. 4.1a,b), no single factor has been demonstrated to provoke a level of stimulation equal to fire (Gill & Ingwersen 1976, Taylor *et al.* 1998). The initiation of flowering in other species, through the application of smoke (Imanishi *et al.* 1986, Keeley 1993) has received considerable attention, instigating experimental work involving exposure to ethylene (Gill & Ingwersen 1976, Keeley 1993), a constituent of smoke (Jacobsen and Gill, unpublished, in Gill 1981a) and often formed as a consequence of stress or injury (Mohr & Schopfer 1995, Hongo & Oinuma 1998, Barker 1999). However, because some species respond positively to smoke, but not to ethylene (Keeley 1993), and dependence of the response on exposure time (Imanishi *et al.* 1986), isolation of the exact combination of factors involved has not been achieved.

Fig. 4.1 (a) Thick smoke generated from the smouldering skirt saturates the crown of this grasstree (1.8 m tall) during a prescribed burn in banksia woodland, potentially exposing the apex to ethylene gas; a known stimulant for inflorescence production (Gill & Ingwersen 1976). (b) The long spike (2 m) covered in creamy-white flowers, supported by the short scape comprises the inflorescence of *X. preissii* (photograph courtesy of R. Wittkuhn).

X. preissii typically flowers in spring (July-November, Marshall 1986), despite suggestions that the species flowers erratically (Leonard 1989. Fig. 4.1b). The typically long and weighty inflorescence has been reported to exceed 4 m in length (Colwill 1999). A small grasstree (*X. preissii*, < 1 m tall) that was recently brought to my attention produced an inflorescence 5.53 m long (W. Kendrick, Witchcliffe resident, pers. comm., Beacham 2001)! To produce such a large structure during one reproductive season is remarkable given the well-deserved, slow-growing reputation of these plants (Gill & Ingwersen 1976, Lamont & Downes 1979). Lamont and Downes (1979) provide a likely annual vertical growth rate estimate of 10–20 mm for *X. preissii*, which is supported by Ward *et al.* (2001). In stark contrast, inflorescence elongation has been reported to be as rapid as 10 cm day^{-1} for a single individual of *X. hastilis* (Cleland 1913). Staff (1976) recorded an inflorescence elongation rate of 7 cm day^{-1} maintained

over one week for *X. australis*, attaining maximum inflorescence length (up to 3 m) after about 75 days.

The advantage of producing a tall conspicuous inflorescence to improve pollinator attraction and resulting pollination success (Lortie & Aarssen 1999) is tempered by the associated costs involved (Stephenson 1981, El-Kassaby & Barclay 1992, Karlsson *et al.* 1996, Obeso 1997). Understandably, costs are relative to the size of the floral display and subsequent seed crop (Gross 1972), and therefore would be exacerbated for grasstrees with more than one head, each producing an inflorescence (Fig. 4.2). Among other things, forced trade-offs between fecundity (seeds), and survival and growth (seedlings) are considered to be costs carried by reproduction (Silvertown & Dodd 1999). For grasstrees reproducing after fire, the costs of reproduction might be compounded with those associated with survival and recovery of prefire plants in the postfire environment. Often mechanisms exist which reduce the impact of these costs. For example, carbon fixation by photosynthetic floral components can balance respiration and contribute to the carbon investment associated with setting seeds (Heilmeier & Whale 1987, Laporte & Delph 1996). Also, Ashman (1994) demonstrated how significant amounts of nitrogen and phosphorus are reabsorbed from senescing floral structures of the perennial plant species *Sidalecea oregana* ssp. *spicata*, mitigating the cost of reproduction.

Fig. 4.2 A 1.6–m tall, 14–headed grasstree (*Xanthorrhoea platyphylla*) on the summit of Bluff Knoll in Western Australia, with 12 heads supporting an inflorescence. In this particularly energetically expensive case, a trade-off between inflorescence number and size is likely.

During the production of large inflorescences, as in *X. preissii,* carbon expenditure is likely to be the most significant reproductive cost. Carbon demand is met from two sources: carbohydrates produced daily through photosynthesis and resources assimilated in previous years and stored in perennial tissues. Like *X. australis* (Staff 1989), *X. preissii* exhibits sympodial growth, as the vegetative apex becomes the site of inflorescence development during reproduction. Carbohydrates derived from carbon fixation, used to support foliage growth in the vegetative state, probably become the main energy source for the young elongating inflorescence. In the Mediterranean-climate zone of Western Australia, many resprouting species rely on starch reserved in specialised storage organs to support reproduction and regeneration following fire (Pate & Dixon 1981, Pate *et al.* 1990, Bowen & Pate 1993, and Chapter 3). Starch is the most important storage carbohydrate in higher plants, and is deposited as insoluble, compact, semi-crystalline particles (starch grains) (Mohr & Schopfer 1995). The presence of starch has been noted in a number of monocotyledons distinguished by having secondary vascular tissue (Tomlinson & Zimmermann 1969), including the genus *Xanthorrhoea*. Staff and Waterhouse (1981) indicated the presence of starch grains in the secondary thickening meristem of *X. australis*, but did not attribute a function to their presence. Even if one was to establish the role of this starch during reproduction, the relative importance of the starch versus foliage-derived assimilates is a further question not commonly addressed in studies of reproductive energetics.

Research addressing reproductive costs often neglect to consider the cost associated with increased water demands created by the growth and maintenance of large inflorescences and flowers. Water loss from floral structures can be substantial, leading to greater water stress and potentially limiting reproductive performance (Whiley *et al.* 1988). Under dry conditions, reduced photosynthetic capacity of plants displaying large flowers, which may negatively affect growth, has been linked with stomatal closure to maintain turgor by preventing water loss (Galen *et al.* 1999).

Inflorescence growth and the later setting of fruits by *X. preissii* is a seemingly energy-expensive process, usually occurring within a short time of fire. The energy and water demands of these plants while reproducing during this critical postfire period must impact their growth, interrupting resprouting progress. Following reproduction, grasstrees are left in a potentially vulnerable state: possibly depleted of stored resources

and returning to a vegetative existence in late-summer when water availability is at a yearly low (Wallace 1965, Beard 1984). I predicted that following further disturbance, such as herbivore damage (McNee 1997) or even after the unlikely event of a second patchy fire (Martin 1966, Lamont & Downes 1979, Jeffrey 1987, Abbott 2002), *X. preissii* would suffer a reduction in leaf growth, reflecting the depletion of stored energy.

This chapter explores these possibilities through an investigation of vegetative and reproductive growth, water status, and the energy resources available to *X. preissii* during reproduction. Specifically, the contribution made by three sources of energy to the production of the inflorescence and fruits following fire were studied. Firstly, it was hypothesised that carbohydrates synthesised by the foliage on a daily basis are redirected from production and development of new leaves to inflorescence elongation. Secondly, starch stored in the desmium of *X. preissii* is consumed as an essential energy source during reproduction. Lastly, as the inflorescence grows beyond the surrounding young leaves it becomes green, suggesting a capacity for photosynthesis. Given the sheer size of this organ it was predicted that it significantly contributes to its own growth and maintenance, through the provision of its own assimilated carbon.

In Chapter 3, the implications of season of burn to the reproductive success of *X. preissii* were outlined. In this chapter, this topic is addressed more fully. *X. preissii* can be burnt as late as early winter and still flower in the following spring. However, the percentage of grasstrees that do this is very low compared with the percentage that flower in response to spring or autumn fires (Lamont *et al.* 2000). For the winter burnt plants that do reproduce in the first postfire spring, little time exists in which to resprout a new crown, possibly jeopardising inflorescence growth and seed development. This interpretation was investigated.

4.2 Materials and methods

4.2.1 Effect of reproduction on leaf production, leaf mass and water relations

A jarrah forest site in the Darling Range (MSB98, refer to Table 1.1) and a banksia woodland site on the Swan Coastal Plain (YSB98, refer to Table 1.1) were chosen for this work. Both sites were burnt in the spring of 1998 and all work was replicated at each site. The study was conducted from 1 September 1999 to 18 April 2000 timed to include the reproductive phenology of *X. preissii*. Distinguishing reproductive grasstrees from vegetative ones before the emergence of the young inflorescence proved to be impossible, a problem also encountered by Staff (1976). For this reason six grasstrees from the jarrah forest site and seven from the banksia woodland site, with small inflorescences (under 85 cm tall), were selected to monitor leaf production, inflorescence elongation and phase of reproduction. A further six grasstrees, assumed to be vegetative (in time this was confirmed by the lack of an inflorescence), were selected from both sites for comparison. All plants were similar in size. Including the inflorescence, each grasstree was sprayed until saturation (the plant was dripping with the solution) every 10 days with a 1.3% solution (v/v in water) of a commercial, non-systemic, broad-spectrum, synthetic, pyrethroid insecticide (Mavrik ®, Sandoz Ltd., Basle, Switzerland and distributed by Arthur Yates and Co. Ltd, NSW, Australia). This treatment was intended to protect developing inflorescences from the boring larvae of the moth, *Meyriccia latro* (Common 1990), previously cited and synonymous with *Hyaletis latro* (Edmonds & Specht 1981) and *Hylaletris latro* (Staff & Waterhouse 1981). Several larval specimens of this species were housed in a rearing chamber at room temperature, to permit the collection and identification of the adult moth (Brian Heterick, entomologist, Curtin University, pers. comm.). Previous studies on unrelated native plants have used both systemic (Krupnick & Weis 1999) and non-systemic insecticides (Zammit & Hood 1986, Wallace & O'Dowd 1989, Vaughton 1990). However, the majority has argued for use of the latter. Non-systemic insecticides are more effective against Lepidoptera and effectiveness is not reduced by plant size or woodiness (Zammit & Hood 1986). Although non-systemic insecticides are less likely to influence plant performance (Louda 1984), the effect of Mavrik on leaf production and growth was tested (refer to Section 4.2.1.3 below) to confirm absence of confounding factors.

4.2.1.1 Leaf production and inflorescence elongation and the phase of reproduction

At the start of the monitoring period the innermost leaves of each selected grasstree were tagged with electrical cable markers (Critchley Pty Ltd, Castle Hill, Australia), the length of the young spike measured and the health of the inflorescence described. Both sites were visited every 15–38 days. On each repeat visit the number of new leaves produced (number of leaves smaller than those tagged previously), length of spike, health of the inflorescence and reproductive phase was recorded for each plant. Grasstrees damaged by grubs were omitted from the study, and when available, a nearby grasstree exhibiting a similar phase of reproduction was substituted into the sample.

4.2.1.2 Leaf mass and water relations

On three occasions over the reproductive season in both habitats, September (16 for jarrah forest and 10 for banksia woodland), December (17 and 23 respectively) and March (4 and 6 respectively), xylem water potential (Ψ_X) was measured predawn and midday for six reproductive and six vegetative, randomly selected grasstrees. Ψ_X was measured on one intermediate leaf (refer to Section 2.2.5.1 for method) from each plant using a pressure chamber (custom-built for Curtin University of Technology).

At both sites, predawn inflorescence water content (WC) was measured on 6–10 randomly selected grasstrees and leaf dry weight was determined for three leaves collected from six reproductive and six vegetative grasstrees. A 10 cm sample was sawn from the middle of each spike and immediately sealed in an airtight plastic bag. Fresh weight was determined by subtracting the air-dried bag weight from the weight of the sealed bag containing plant material. Dry weight was measured after plant material was dried at 70 ˚C for 48 h or constant weight. WC was calculated as a percentage of fresh weight:

$$\text{WC (\%)} = (\text{fresh weight} - \text{dry weight})/\text{fresh weight} \times 100$$

4.2.1.3 Testing the effect of insecticide on leaf production and growth

Mavrik is designed to control a variety of chewing and sucking insects by contact (active constituent 7.5 g/L Fluvalinate). Eight grasstrees from the two unburnt control sites on the Darling Range and on the Swan Coastal Plain (MC and YC; refer to Table 1.1) were

used to test the effects of Mavrik. Over a period of 110 days coinciding with the growing season (1 September to 20 December) four of the plants were sprayed until saturation every 10 days with a 1.3% solution (13 ml Mavrik in 1 L of water) and the remaining four were sprayed with the same quantity of deionised water. Leaf production, young leaf elongation, intermediate leaf elongation and mature leaf death were recorded (refer to Section 2.2.2 for details of method).

4.2.2 Effect of season of burn on inflorescence and seed biomass

At two sites on the Swan Coastal Plain (YAB00, burnt in autumn 2000 and WSB99, burnt in spring 1999; Table 1.1) 15 inflorescences produced in spring 2000 were harvested (on 15 February 2001 and 19 February 2001 respectively). Inflorescences were selected on a random walk through the study site by the observer, omitting any that appeared to have suffered grub damage. Each inflorescence was cut into short sections and placed in a labelled paper bag. All bags were dried at 65 ˚C for one week or until constant weight. Dry mass of the scape and spike was determined separately after remaining seeds had been shaken loose. A sample of 1–20 oven dried seeds was weighed for each spike holding seeds (10 spikes from YAB00 and 13 spikes from WSB99) to determine mean seed mass.

4.2.3 Effect of reproduction on caudex starch reserves

This work was developed from a preliminary investigation that revealed a visual reduction of desmium starch in grasstrees that had flowered compared with their vegetative counterparts (Fig. 4.3a,b). Burnt in spring 1999, the site chosen for this work (WSB99; Table 1.1) was typical of banksia woodland on the Swan Coastal Plain. On four occasions samples of desmium tissue were collected from the caudex of four vegetative and four reproductive grasstrees, by drilling through the layer of leafbases on the stem and using a cork corer to remove desmium (refer to Section 3.2.5.1 for detail). Grasstrees were sampled between 11 September 2000 and 16 February 2001, during critical stages of reproduction: inflorescence < 0.5 m, inflorescence > 2 m, inflorescence with swollen fruits, and seeds released. Desmium samples were hand-sectioned, stained with 2% iodine solution (1 KI : 1 I_2 by weight in water) and then mounted in a water/glycerol medium for examination under a dissecting scope in order to determine

the starch index (a relative measure of the status of the starch reserves). A detailed description of the preparation and analysis technique is given in Section 3.2.5.1.

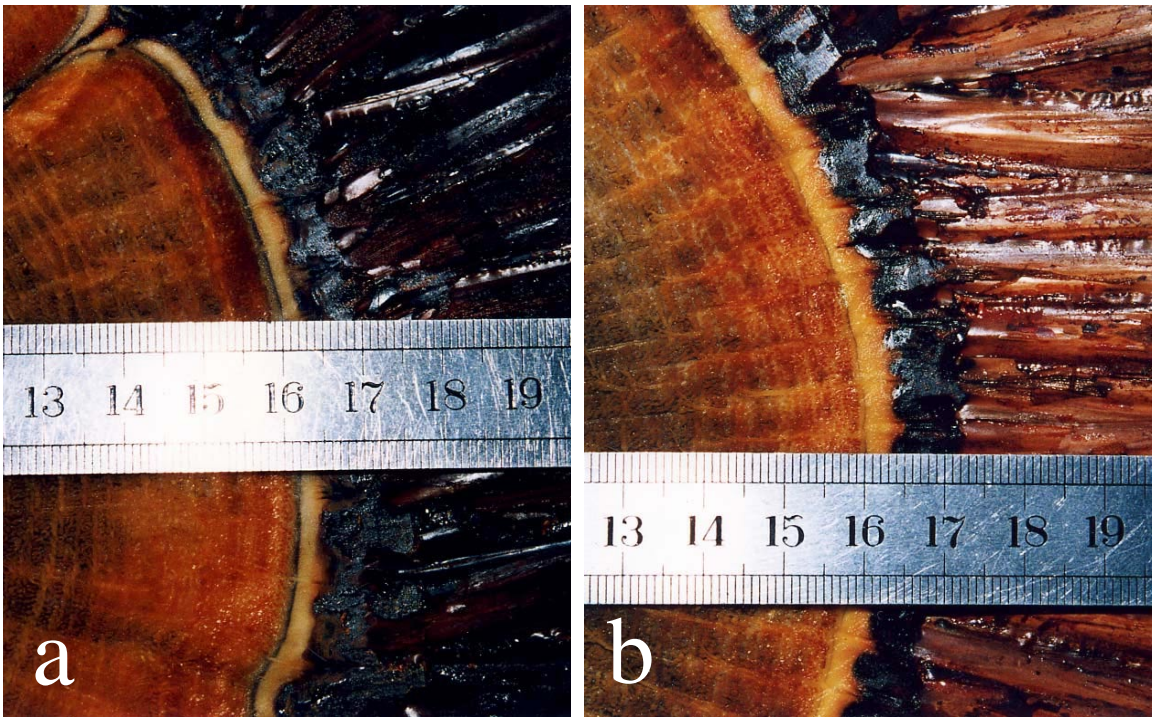

Fig. 4.3 A transverse section through the stem of a (a) vegetative and a (b) reproductive grasstree. Staining with iodine highlights the location of starch (the fine, dark line at the 16 cm mark) in the desmium tissue, which separates the leafbases from the inner fibrous core. Note the difference in the width of the line of starch between the two sections.

4.2.4 Experiment 1: The effect of leaf removal following reproduction on further leaf production

The jarrah forest spring-burnt site (MSB98; Table 1.1) was chosen for the purpose of this field-based experiment. The study commenced on 3 February 2000 at the time of seed release from the plants. A further six plants, at a similar stage of reproduction, were added to the grasstrees used for leaf production and inflorescence monitoring (refer to Section 4.2.1.1), increasing the sample to 12. Three of the new and three of the original grasstrees were randomly allocated to a leaf clipping treatment while the remaining plants were monitored as controls. The six control plants were left intact with the smallest innermost leaves tagged (as described in Section 4.2.1.1) and the remaining six had the existing green foliage removed using secateurs, leaving only the old, dry flowering spike. During the experiment cylindrical wire cages were placed over each plant crown to prevent kangaroo grazing. Light interception by the cages was assessed by measuring photosynthetically active radiation (PAR) above the crown of all

grasstrees, with and without the cage protection, using a quantum sensor (model LI-185B, Li-Cor Inc., Lambda Instruments Corporation, Nebraska, USA). These measurements were made within a 45–min period over midday. The site was visited every 14 to 38 days, when the number of new leaves produced by all grasstrees was recorded. At the end of the experiment, on 26 July 2000, the desmium tissue of each grasstree was sampled for starch reserve analysis (starch index determination; Section 3.2.5.1).

4.2.5 Experiment 2: The relative significance of grasstree energy sources to inflorescence production

Locating a site with sufficient individuals of *X. preissii* that were in the early stages of inflorescence elongation was critical to the success of this experiment. The banksia woodland site burnt in spring 1999 (WSB99; Table 1.1) met this requirement. By walking through the site examining all single-stemmed plants encountered, 28 grasstrees with emerging young inflorescences were located, marked with flagging tape and mapped. For each grasstree, inflorescence dimensions (length and circumference) and health (score 1–5; where 5 was light green, fresh in appearance with no grub damage, and 1 was brown markings, dry tip or grub damage) were recorded, as well as plant height and stem circumference 0.4 m from the ground. Twenty plants with the shortest (youngest) inflorescence and highest health score were selected from the sample. This allowed a complete study of treatment effects, independent of grub or other damage.

Each plant was allocated to one of four 'covering' treatments (4 September 2000): foliage only covered, inflorescence only covered, both foliage and inflorescence covered and no cover (Fig. 4.4a–c). Foliage was covered using two layers of 90%, woven green shade cloth (Coolooroo®, Melbourne, Australia) loosely draped over the grasstree and secured at the base of the stem with rope. Inflorescence cover consisted of a custom-made pale green polyester-cotton sock, which slid down over the spike and pushed tightly into the surrounding foliage where the scape arose. Both cover types met the requirements of a sufficiently loose weave to allow some air circulation while reducing photosynthetically active radiation (PAR) to less than 5 % (Table 4.1). Fitzpatrick and Nix (1970) estimated that the relative dry matter production under this light regime was < 12%. PAR was measured for open sun and under the two cover types using a quantum sensor (model as above). Mavrik was applied to all inflorescences before covering and

Fig. 4.4 Four treatments applied to grasstrees during a manipulative experiment aimed at determining the relative significance of grasstree carbon sources to inflorescence production. (a) young inflorescence growing through a small opening in the foliage cover treatment. (b) sock-like inflorescence cover concealing a full-length inflorescence. (c) *X. preissii* subjected to both foliage and inflorescence cover, and (d) an example of the control treatment, "no cover". Grasstrees are 0.7–1.0 m tall.

repeatedly applied to all plants in accordance with the manufacturer's instructions (solution of 13 mL Mavrik in 1 L water, every 10 days). The site was visited every 5 to 13 days during which inflorescence length was measured and inflorescence health recorded. Two plants were replaced within a short time of commencing the experiment due to severe grub damage, despite insecticide treatment. Once elongation had ceased and the plant was in flower (14 November 2000), all inflorescences were harvested, cut into small lengths and stored in labelled paper bags. Due to the high moisture content and density of the material each bag was dried for one week at 65 °C or until constant weight before biomass could be determined. Samples of desmium tissue were also collected from each grasstree and analysed for relative starch content (starch index; method described in Section 3.2.5.1).

Table 4.1 Photosynthetically active radiation (PAR) measured in the open and under both treatment cover types. PAR is given as the mean ± SE with *n* in parentheses, and is also expressed as a percentage of full sunlight.

Cover type	PAR (μmol m^{-2} s^{-1})	PAR in full sunlight %
None (full sunlight)	1089.0 ± 184.4 (10)	100.0 ± 0.0
Foliage cover	4.6 ± 0.8 (5)	0.7 ± 0.2
Inflorescence cover	25.6 ± 7.2 (5)	2.3 ± 0.6

Using the following procedure, an estimate was made of the mass of total carbon, in the form of starch, available to the developing *X. preissii* inflorescence. The inflorescence mass and starch index of the reproductive grasstrees subjected to both cover types, and also the starch index of four similar-sized vegetative plants, sampled one week later, was used. The starch index for each reproductive grasstree (SI_{REPROD}) was divided by the mean starch index for the vegetative grasstrees (SI_{VEG}) to give the fraction of starch remaining (Sp_{REMAIN}) after inflorescence production.

$$Sp_{REMAIN} = SI_{REPROD}/SI_{VEG}$$

The proportion of starch used (Sp_{USED}) to produce the inflorescence was ($1 - Sp_{REMAIN}$). It was assumed that the final inflorescence mass of those grasstrees with both foliage and inflorescence covered was derived from carbon stored as starch in the desmium, and a relatively small fraction from protein and mineral nutrients. Witkowski and Lamont (1996) calculated that mineral nutrients comprised less than 2% of the dry mass of the

flower heads and canopy leaves of *Banksia hookeriana*. Assuming a similar relationship for *X. preissii*, it follows that the mass equivalent of Sp_{USED} is equal to the inflorescence mass (M_{INFL}) minus 2% (mineral nutrients), but added to this is the mass of starch required for the energetic costs of starch hydrolysis and the subsequent synthesis of tissues. To estimate this allowance, data from Villar and Merino (2001) was used. In their study, a calorimetric method was employed to calculate the leaf construction cost for several chaparral and mediterranean forest woody species from Spain and California as 1.5 g glucose/g tissue mass (ie 50% cost). As the pathway to convert stored starch to tissue requires only 12.5% of the total ATP equivalence of energy used to produce tissue via the fixation of CO_2 (collated from Salisbury & Ross 1992), 13% of the portion of the construction cost allocated to synthesis of tissue by Villar and Merino was considered reasonable to account for the respiratory costs of grasstree inflorescence production (ie 6%). Therefore, to estimate the total mass of starch available (M_{AVAIL}) to *X. preissii* before reproduction the mass equivalent of Sp_{REMAIN} was added to M_{INFL}, in addition to the further 6% for respiratory costs, minus 2% to account for the mineral nutrient contribution (ie 100% + 6% – 2% = 104%).

$$M_{AVAIL} = ((Sp_{REMAIN}/Sp_{USED} \times M_{INFL}) + M_{INFL}) \times 1.04$$

Leaf and inflorescence surface temperatures were measured for each plant on a typical fine, sunny day for the time of year (22 September 2000). Temperatures were recorded hourly from 11:00 to 14:00 (hottest part of the day) using an infrared field thermometer (model PRT-10L, Barnes Engineering Company, Connecticut, USA). For comparison, to assess the likelihood of grasstrees overheating under covering treatments, mean maximum daily temperature was obtained for the period of highest leaf production (see Fig. 2.6b in Section 2.3.1.1 for details regarding leaf production) for grasstrees from the YSB98 site one year after fire: the same timing since fire as for the site WSB99, used in this experiment.

Comparing relative fluorescence values with those for the foliage was used as a novel approach to detect the ability of the inflorescence to photosynthesize. Fluorescence was measured for each leaf category (see Section 2.2.2 for a description of leaf categories) and inflorescence (scape and spike separately), after a 20–min dark adaption period,

using a fluorescence meter (Plant Stress Meter Mark II, Biomonitor AB SCI, Umea, Sweden). Measurement of relative fluorescence (F_v/F_m) was made, which is given by:

$$(F_m - F_o)/F_m = F_v/F_m,$$

Where F_m = maximum fluorescence, F_o = base level fluorescence, and F_v = maximum variable fluorescence.

4.2.6 Statistical analysis

All statistical data analysis was performed using SPSS 10.0 software program (SPSS Inc., Chicago, USA). Examination of residual stem-and-leaf plots, as well as the calculation of the Shapiro-Wilks statistic, was used to test for normality. Levene's test was employed to test for homogeneity of variances. Based on the outcome of this data screening some data were square root or log transformed to meet the assumptions of each statistical test. All transformed data are presented as untransformed means. In the case where data transformation did not improve variance homogeneity a non-parametric test was used.

4.3 Results

4.3.1 Leaf and Inflorescence growth

As early as March, inflorescence primordia were noted buried among the leaves surrounding the apex during the dissection of grasstrees burnt the previous spring. At both sites inflorescence elongation was first identified in late August/September reaching a maximum growth rate during October, before ceasing in late November (Fig. 4.5). Despite grasstrees from the two habitats having a similar phenology, spike elongation rates in banksia woodland were consistently greater, resulting in a taller

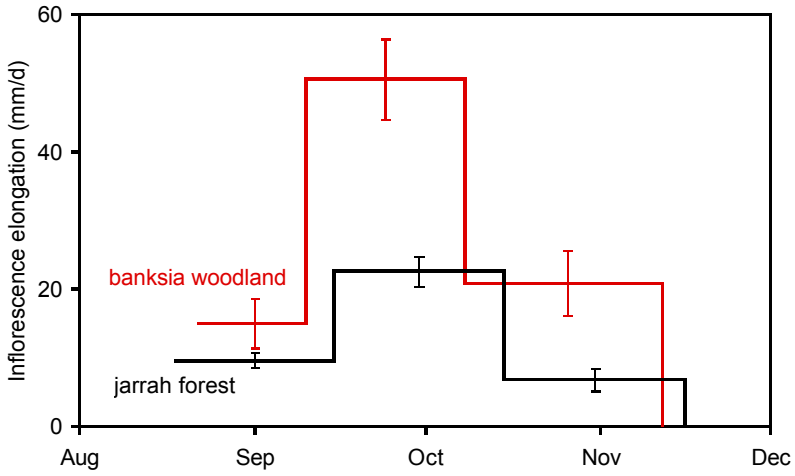

Fig. 4.5 Inflorescence elongation rate of grasstrees growing in jarrah forest and banksia woodland measured during 1999. Data are the mean of 5–6 plants for each measurement period (horizontal line) ± SE.

mature spike (2.7 ± 0.4 m (mean ± SD), contrasted with 1.3 ± 0.3 m for jarrah forest inflorescences). During spike production and maturation, leaf production was greatly reduced at both sites (Fig. 4.6a,b). Initially, the rates of leaf accumulation (expressed as the slope of the lines in Fig. 4.6a,b) for vegetative and reproductive grasstrees slowly diverged, but increased markedly following maximum spike elongation. Leaf accumulation by the reproductive plants in both habitats demonstrated little sign of recovery until after seed release in late January/early February. Over the period of reproduction, the vegetative leaf accumulation rate was 4.6 times greater in jarrah forest and 3.0 times greater in banksia woodland than the corresponding rates for reproductive plants. This difference in leaf production rate accounted for an average production of 216 and 239 extra leaves by the vegetative grasstrees in the jarrah forest and banksia

woodland respectively. These values represent 47 and 40% of the mean annual leaf production for the vegetative grasstrees in jarrah forest and banksia woodland respectively. An independent *t*-test was used to determine if a site difference existed for these results, deriving individual percentages (then arcsine transformed) from random pairing of reproductive and vegetative grasstrees. The 7% difference between the sample means was not significant ($P = 0.530$).

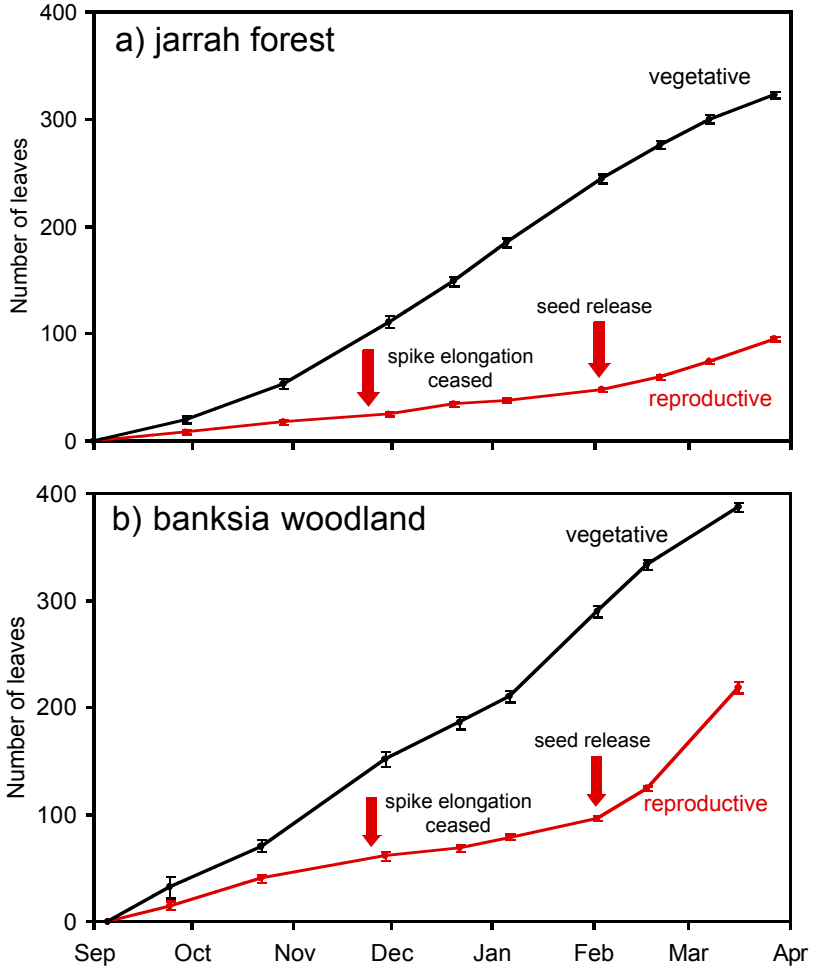

Fig 4.6 Rate of leaf accumulation by vegetative and reproductive grasstrees (1999–2000) in (a) jarrah forest and (b) banksia woodland. The timing of significant stages of reproduction is indicated. Data are the means of 5–6 plants ± SE.

Commonly associated with early inflorescence growth, particularly in the banksia woodland, was the formation of deformed or flattened leaves (Fig. 4.7). Reproductive grasstrees exhibited a flatter top, instead of their typically globular crown, due to the stunted growth of the associated leaves (Fig. 4.7). Stunted leaf growth in reproductive grasstrees was also indicated by a lower dry leaf mass than their vegetative counterparts in jarrah forest and banksia woodland (Table 4.2). This difference was clear after the

Fig. 4.7 (Left) deformed leaves (3 on left) collected from near the apex of a grasstree with a young inflorescence, and equivalent aged normal leaves (3 on right) from a vegetative grasstree. Scale in cm. (Right) a grasstree apex ground down to the leafbases to reveal an old inflorescence scape (about 2.5 cm diameter) adjacent to the new apical meristem (photograph courtesy of B. Lamont). Note the reduced size of the leafbases that would have been coincident with the timing of the flowering event, contrasted against the normal size of the new leaves around the new apex.

Table 4.2 Intermediate leaf dry mass (g/leaf) for vegetative and reproductive grasstrees in jarrah forest and banksia woodland, over the period spring 1999 to autumn 2000. Data are means of six plants. The non-parametric Friedman test indicates significance between vegetative and reproductive grasstrees in jarrah forest, and two-way ANOVA compares the two plant categories over time in the banksia woodland.

	Jarrah forest		Banksia woodland	
Time	Vegetative	Reproductive	Vegetative	Reproductive
September	0.486	0.347	0.890	0.936
December	0.508	0.376	1.087	0.956
March	0.430	0.273	0.979	0.674
Two-way ANOVA				
Lifecycle phase (L)			$P < 0.0001$	
Time (T)			$P < 0.0001$	
L × T			$P < 0.0001$	
Friedman test	$P = 0.029$			

first measurement in September in jarrah forest, and after the December measurement in banksia woodland. Leaf mass for the banksia woodland grasstrees varied over time and there was an interaction effect between lifecycle phase and time. The latter was a consequence of both vegetative and reproductive grasstrees having equivalent leaf mass during September, if not marginally lower for the vegetative plants.

4.3.2 Water relations

Mean inflorescence water content (WC) measured at predawn was consistent for *X. preissii* in jarrah forest and banksia woodland (Fig. 4.8). Initially starting at 81–83 % during early September, inflorescence WC dropped to 70–71 % three weeks after the peak of flowering. Predawn and midday Ψ_X was not significantly different over time. However, a significant difference existed between sites (Table 4.3), with jarrah forest

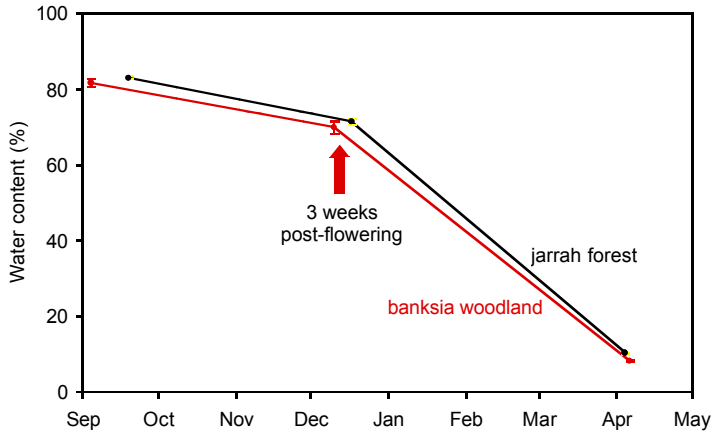

Fig. 4.8 Inflorescence water content, expressed as a percentage of fresh weight, for *X. preissii* in jarrah forest and banksia woodland during the 1999/2000 reproductive season. Data are means of 6–10 plants ± SE.

Table 4.3 Three-way ANOVA summary of xylem water potential for *X. preissii* during vegetative and reproductive phases, at three times (September 1999, December 1999 and March 2000) over the reproductive season, and in jarrah forest (MSB98) and banksia woodland (YSB98).

	P Predawn	*P* Midday
Lifecycle phase (L)	0.431	0.002
Time (T)	0.621	0.834
Site (S)	0.001	0.008
L × T	0.686	0.862
L × S	0.594	0.965
T × S	< 0.0001	0.016
L × T × S	0.590	0.736

grasstree Ψ_X marginally lower (Fig. 4.9a,b). The lowest (most negative) predawn Ψ_X was –0.94 MPa and the lowest midday was –2.25 MPa, although most measurements were considerably higher (Figure 4.9a,b). Predawn, no significant difference existed between vegetative and reproductive Ψ_X, but the reproductive and vegetative midday Ψ_X, illustrated in Figure 4.9b, were significantly different (Table 4.3).

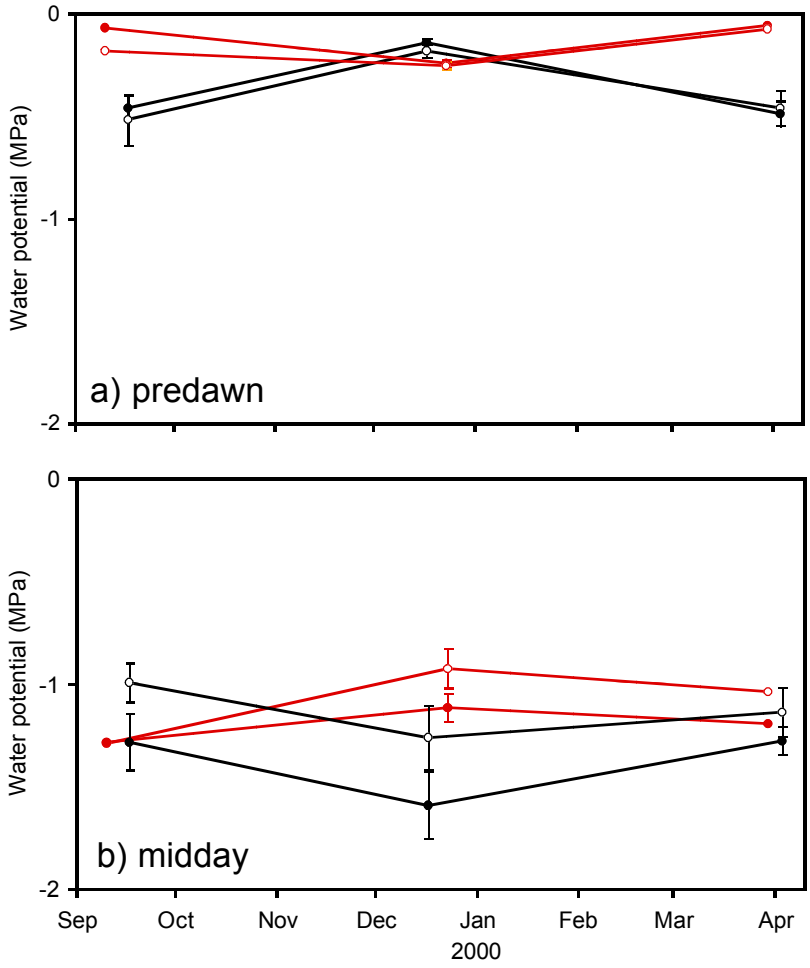

Fig. 4.9 Xylem water potentials measured (a) predawn and (b) midday on vegetative (solid) and reproductive (open) grasstrees in jarrah forest (—) and banksia woodland (—) over the 1999–2000 reproductive season. Data are means of six plants ± SE.

4.3.3 Testing the effect of insecticide on leaf production and growth

All measures of leaf production, elongation and death indicated no significant difference between the performance of grasstrees sprayed with Mavrik and those sprayed with deionised water (Tables 4.4 and 4.5). Furthermore, the only significant results demonstrated that both sprayed and unsprayed grasstrees experienced a consistent change in leaf progression over time (Table 4.5).

Table 4.4 Leaf production rates (mean ± SE leaves/d, and $n = 4$) of grasstrees during the growing season (spring/summer) either sprayed with Mavrik or not sprayed, and growing in either jarrah forest or banksia woodland. *P*-values indicate the results of a two-way ANOVA conducted for each habitat.

	Jarrah forest				Banksia woodland			
Time	September	October	November	December	September	October	November	December
Leaf production								
Sprayed	0.93 ± 0.13	0.73 ± 0.04	1.11 ± 0.29	1.21 ± 0.24	0.63 ± 0.15	0.68 ± 0.18	0.46 ± 0.10	0.61 ± 0.24
Not sprayed	1.04 ± 0.33	1.10 ± 0.21	1.10 ± 0.37	1.33 ± 0.25	0.75 ± 0.08	0.64 ± 0.24	0.88 ± 0.33	0.50 ± 0.14
Two-way ANOVA								
Spraying (S)			0.643				0.623	
Time (T)			0.165				0.856	
S × T			0.675				0.425	

Table 4.5 Elongation rates (% leaves progressing to the next leaf category per day) of young and intermediate leaves, and leaf death rate (% mature leaves dying per day, see Section 2.2.2 for further clarification) on *X. preissii* either sprayed with Mavrik or not sprayed. Grasstrees were monitored in both jarrah forest and banksia woodland, during the growing season (spring/summer). Data are mean ± SE, with $n = 4$. *P*-values indicate the results of two-way ANOVA for each habitat.

	Jarrah forest		Banksia woodland	
Time	October	December	October	December
Young → Intermediate				
Sprayed	0.43 ± 0.10	0.75 ± 0.03	0.36 ± 0.12	0.74 ± 0.10
Not sprayed	0.67 ± 0.16	0.67 ± 0.20	0.36 ± 0.10	0.84 ± 0.01
Two-way ANOVA				
Spraying (S)	0.465		0.806	
Time (T)	0.378		0.010	
S × T	0.379		0.695	
Intermediate → Mature				
Sprayed	0.04 ± 0.01	0.29 ± 0.02	0.15 ± 0.03	0.16 ± 0.04
Not sprayed	0.06 ± 0.01	0.28 ± 0.02	0.12 ± 0.01	0.13 ± 0.03
Two-way ANOVA				
Spraying (S)	0.111		0.304	
Time (T)	< 0.0001		0.759	
S × T	0.855		0.978	
Mature → Dead				
Sprayed	0.00 ± 0.00	0.04 ± 0.02	0.00 ± 0.00	0.06 ± 0.02
Not sprayed	0.00 ± 0.00	0.00	0.00 ± 0.00	0.04 ± 0.01
Two-way ANOVA				
Spraying (S)	0.355		0.175	
Time (T)	0.067		0.004	
S × T	0.119		0.314	

4.3.4 Effect of season of burn on inflorescence and seed biomass

Mean inflorescence dry mass was considerably larger for spring-burnt grasstrees than for autumn burnt grasstrees (1.42 ± 0.68 kg (mean ± SD) and 0.95 ± 0.46 kg respectively). This difference could be attributed to the spring burnt plants having a heavier spike, but not scape (Table 4.6). Season of burn did not affect individual seed mass (Table 4.6).

Table 4.6 Mean (± SE) inflorescence spike and scape dry mass (kg) and single dry seed mass (mg) for 15 inflorescences collected from spring (WSB99) and autumn (YAB00) burnt sites. Mean individual seed mass was determined for each infructescence from the seeds collected (sub-samples of 1–20 seeds).

Inflorescence component	Spring	Autumn	*P* (*t*-test)
Scape mass (kg)	0.23 ± 0.03	0.18 ± 0.02	0.171
Spike mass (kg)	1.19 ± 0.62	0.78 ± 0.11	0.039
Single seed mass (mg)	15.12 ± 1.06	12.35 ± 6.67	0.262

4.3.5 Effect of reproduction on caudex starch reserves

During the early stages of inflorescence elongation the starch index (SI) was somewhat lower than that of vegetative grasstrees (Fig. 4.10). By the time inflorescence length exceeded 2 m and anthesis had started, mean SI had risen to values similar to those of vegetative plants. Two sequential decreases in the reproductive grasstree SI coincided with swelling of the capsules, and maturation of the seeds and their subsequent release. Overall, significant differences in SI occurred over time ($P = 0.037$, two-way ANOVA), due primarily to changes associated with the reproductive grasstrees (Fig. 4.10). Additionally, SI for the two plant categories differed significantly ($P < 0.0001$), and vegetative grasstrees appeared to have a higher SI than equivalent reproductive grasstrees.

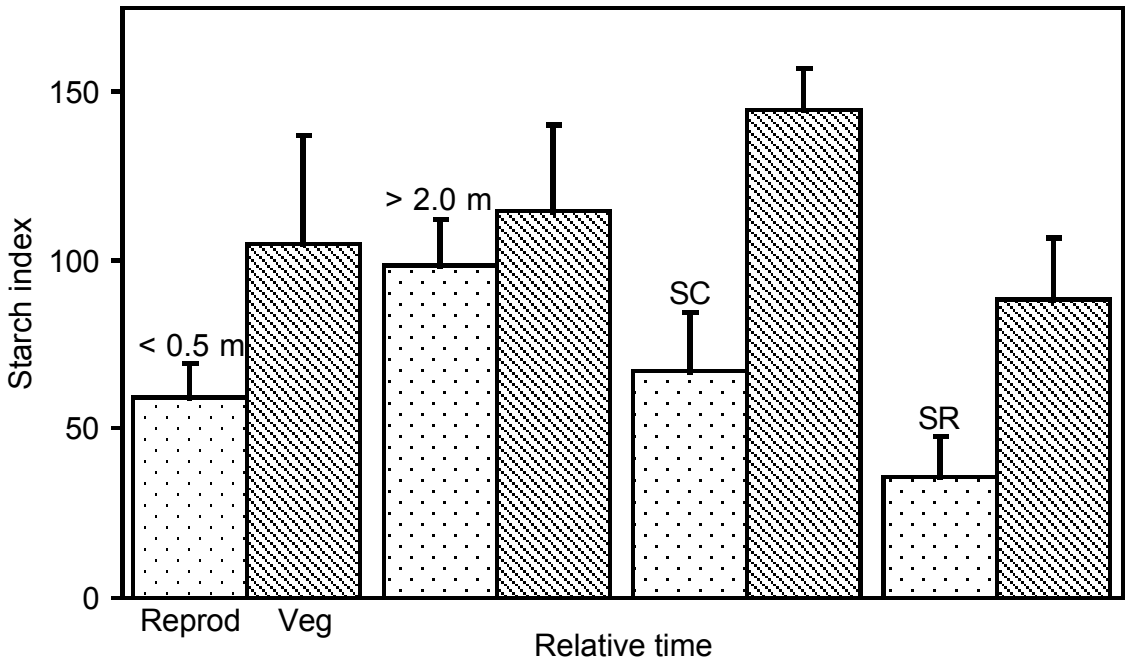

Fig. 4.10 Starch index for (hatched, veg) vegetative and (stippled, reprod) reproductive grasstrees during the 2000/2001 reproductive season. Labels indicate the consecutive phases of reproduction: inflorescence length, SC = presence of swollen capsules, and SR = seeds undergoing release. Data are means of four plants ± SE.

4.3.6 Experiment 1: The effect of leaf removal following reproduction on further leaf production

Photosynthetically active radiation (PAR) was reduced by less than 4.5% when a cage was applied to a grasstree for protection of leaves against grazing (mean PAR (± SE) without cage was 307.7 ± 84.2, and with cage was 298.3 ± 85.0 μmol m^{-2} s^{-1}). Prior to imposing the leaf-removal treatment leaf production rate was identical for both control and treated grasstrees (Fig. 4.11). Clipped plants responded with a large increase in leaf production immediately after the treatment. Initial leaf production rates were elevated to 5.6 times those of intact grasstrees, followed by a gradual decrease to control levels within 3–4 months. Over this period, control leaf production levels were maintained relatively constant, at less then one leaf/d. Clipped plants had a SI of 12.2 ± 4.0 (mean ± SE) compared with 20.1 ± 7.7 for the unclipped plants after 5 months. This apparent difference in SI values was not significant ($P = 0.192$, *t*-test).

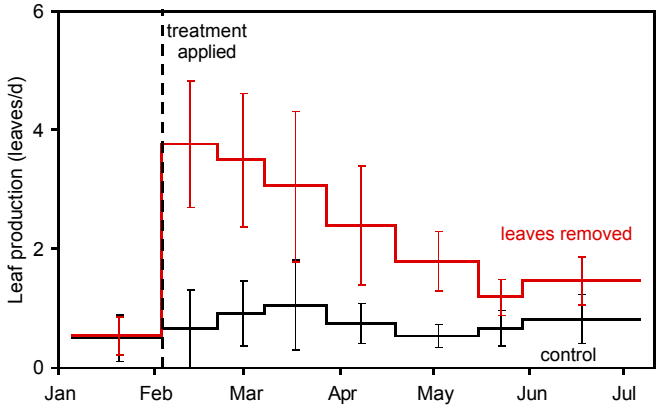

Fig. 4.11 Leaf production rate of grasstrees that had completed reproduction and were then subjected to one of two treatments: entire leaf removal and no leaves removed (control). Measurements were made during 2000 at a jarrah forest site (MSB98). Data are means of six plants ± SE.

4.3.7 Experiment 2: Determining the relative significance of grasstree energy sources to inflorescence production

Relative fluorescence of the three leaf categories and inflorescence scape and spike were significantly different (P = 0.001, one-way ANOVA). Bonferroni multiple comparisons test indicated that this difference was primarily due to the low relative fluorescence of the young leaves (young leaf comparisons with scape, P = 0.002; spike, P = 0.010; mature leaf, P = 0.055; and intermediate leaf, P = 0.001. Fig. 4.12). Also, relative fluorescence of the scape and spike was not significantly different from that of the intermediate leaf category (P = 1.000 for both comparisons; Bonferroni multiple comparison test), which recorded the highest mean relative fluorescence (0.769).

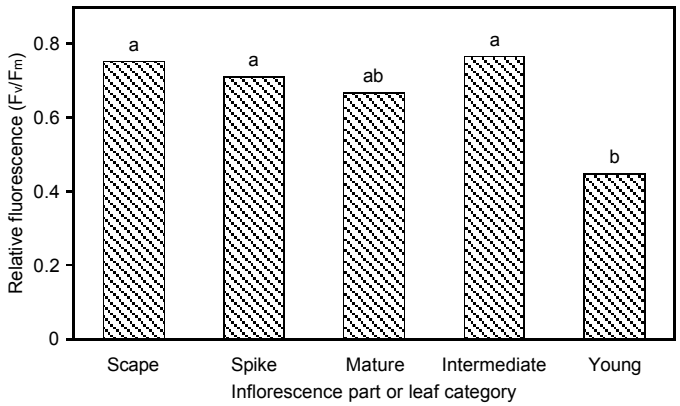

Fig. 4.12 Relative fluorescence of the scape, spike, and the three leaf categories of *X. preissii*, prior to anthesis (see Section 2.2.2 for descriptions of leaf categories), growing in banksia woodland. Lowercase letters indicate significant differences across plant

Leaf and inflorescence temperatures were recorded on a clear day with a maximum air temperature of 22.5 ˚C (Fig. 4.13a,b). Only measurements made when the subject grasstree was in full sun were accepted into the data set, representing maximum possible

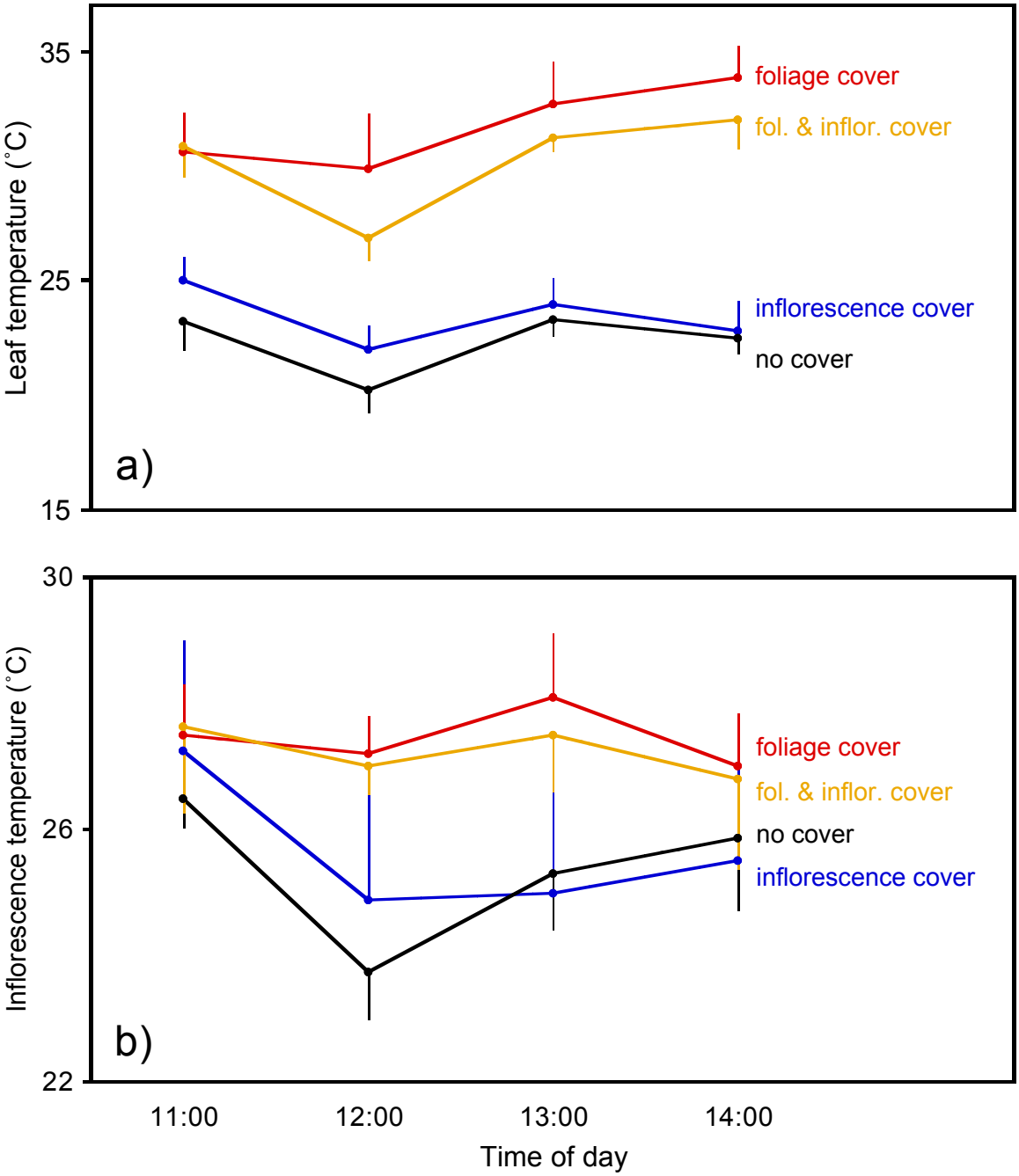

Fig. 4.13 (a) leaf and (b) inflorescence temperatures for banksia woodland grasstrees, over midday, subjected to four combinations of foliage cover, inflorescence cover and no cover. Measurements were made during a fine spring day with maximum temperature of 22.5 ˚C. Data are means ± SE, when grasstrees were exposed to full sun conditions (*n* = 2–5 plants).

temperatures. Leaf temperature of grasstrees subjected to the treatments involving foliage cover was consistently the highest. A three-way ANOVA indicated that leaf temperatures were significantly different over time ($P = 0.004$), and that foliage cover had a significant effect on leaf temperature ($P < 0.0001$). Similarly, inflorescence temperature was significantly higher for grasstrees with foliage covered than those without ($P = 0.004$), but this treatment effect was not significantly different over time ($P = 0.424$). For comparison, maximum air temperature at the equivalent spring-burnt site (YSB98) was 31.9 ± 0.9 ˚C (mean ± SE) during the period of highest grasstree leaf production (from Chapter 3), which was 2 ˚C lower than the highest mean leaf temperature recorded at WSB99 during this experiment.

The slope of each line in Fig. 4.14a depicts the mean inflorescence elongation rate for each treatment. Initial inflorescence elongation rates were similar for all grasstrees, and it was not until after day 21 that a treatment effect became apparent. By day 74, treatment effects were more prominent, with a significant effect of foliage ($P = 0.027$, two-way ANOVA) and inflorescence cover ($P = 0.025$) on inflorescence length by harvest time. Covering foliage had a significant effect on final inflorescence dry mass ($P = 0.001$, two-way ANOVA. Fig. 4.14b), but the effect of inflorescence cover was not significant ($P = 0.170$). The presence or absence of foliage cover also had a significant effect on starch reserves ($P = 0.009$, two-way ANOVA), but again the effect of inflorescence cover was not significant ($P = 0.128$). A reduction in starch reserves occurred when either form of cover was applied during inflorescence production (Fig. 4.14c), but this was most apparent for covered foliage.

Extrapolating from the data collected during this experiment, *X. preissii* had 0.493 ± 0.200 kg of dry mass available as stored starch prior to reproduction. As desmium volume, and therefore potential starch storage, is dependent on grasstree size, this value is representative of grasstrees within the size range studied: circumference 73–100 cm and height 67–101 cm.

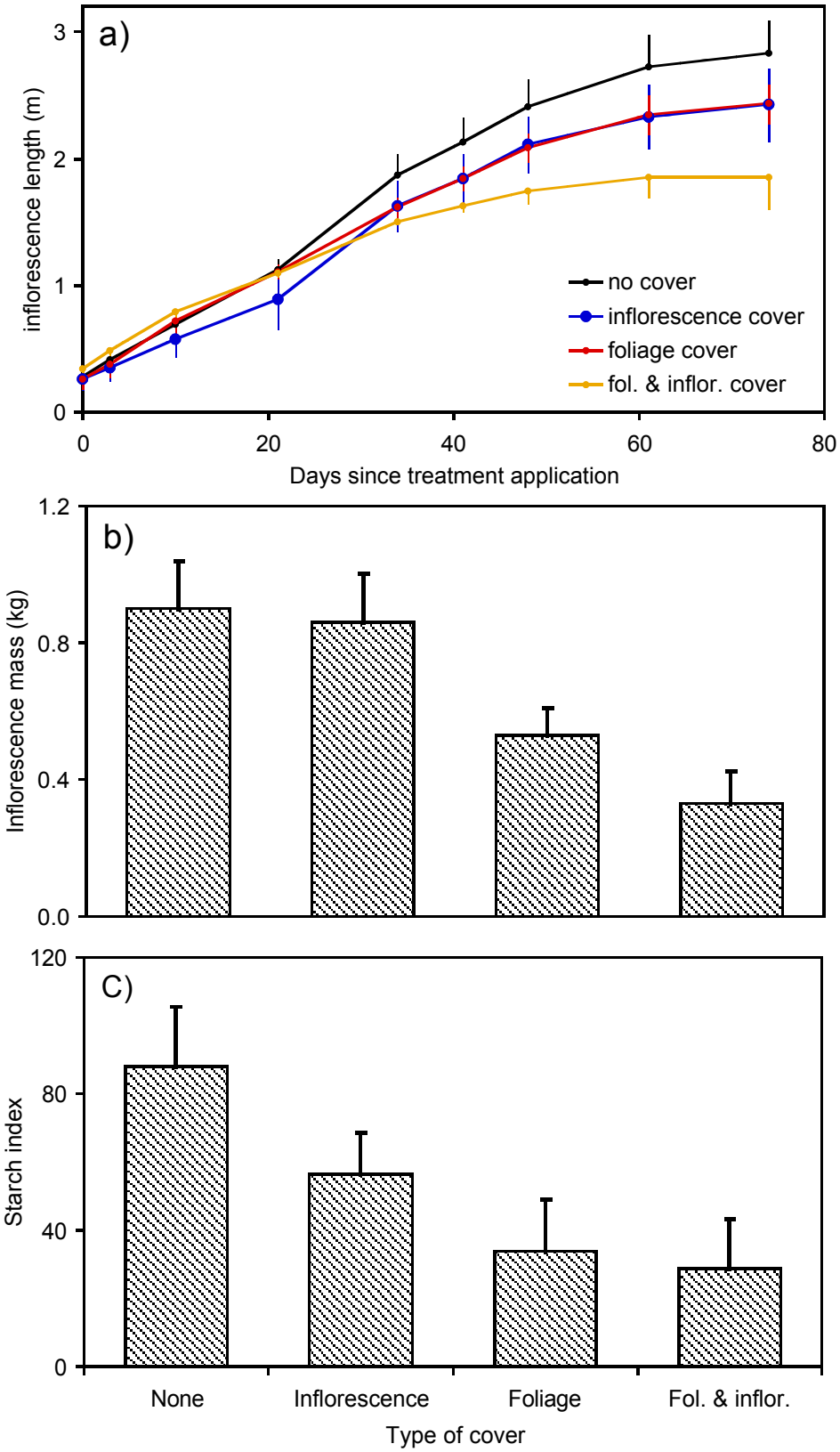

Fig. 4.14 (a) inflorescence length, (b) inflorescence dry mass and (c) starch index of *X. preissii* over time after the application of treatments comprising the four combinations of foliage cover, inflorescence cover, and no cover. Data were collected during the 2000

4.4 Discussion

After fire, most grasstrees underwent what can be considered the most dynamic phase of their lifecycle: accelerated leaf growth (Chapter 3) accompanied by the rapid production of a large inflorescence. On these responses alone it would be fair to say that fire is the most significant single factor to influence the lifecycle of grasstrees. Rapid resprouting by *X. preissii* following fire exceeds that of all other co-occurring species (Baird 1977). Inflorescence production by *X. preissii* is another example of growth beyond the plant's normal output, measured in my study at a mean maximum elongation rate of 5.6 cm d^{-1}; higher maximum growth rates have been reported by other authors for different grasstree species (Cleland 1913, Staff 1976). Variation between maximum inflorescence elongation rates was most likely a reflection of site effects and methodological discrepancies, rather than species differences. For example, Cleland's (1913) highest measurement of 10 cm d^{-1} was made on a single specimen of *X. hastilis* growing in his garden, a highly modified environment, possibly richer in nutrients and subject to greater water availability compared to either jarrah forest or banksia woodland. Also, Staff's (1976) highest measurement of 7 cm d^{-1} was only recorded for one of the 20 grasstrees (*X. australis*) he monitored on a weekly basis, which is comparable to the fastest elongating individual I recorded (6.8 cm d^{-1}) over a four week period.

Not until late August were the foliage-derived carbohydrates redirected away from leaf production towards the young inflorescence primordia replacing the terminal vegetative apex (Staff & Waterhouse 1981) that becomes the new sink for this energy. Leaf production is drastically reduced from the moment the inflorescence starts to elongate in early September, and is maintained until seed is released about five months later. The detrimental effect of reproduction on normal vegetative growth is common in many species (El-Kassaby & Barclay 1992, Obeso 1997, Silvertown & Dodd 1999), and is further exacerbated by abnormally large seed production (Gross 1972). For a grasstree regenerating its crown previously lost to fire, this sacrifice of leaf production is a major trade-off. Since the reproductive and growing seasons overlap, the significance of this cost is further magnified, as the grasstree has also forfeited the opportunity for maximum leaf growth during late spring/early summer when growing conditions are optimal.

Stunted growth in young expanding leaves also indicates the temporary diversion of energy towards the inflorescence (Karlsson *et al*. 1996, Obeso 1997). In jarrah forest, reproductive grasstrees maintained the lowest leaf mass, monitored from the early stages of reproduction when the mean inflorescence height for the sampled plants was < 0.5 m. This suggests that the foliage had been deprived of energy for some time and the redirection of photosynthate pathways occurs very early. In contrast, leaf mass of banksia woodland grasstrees did not show the same sensitivity to early inflorescence growth, evidenced by the similarity of vegetative and reproductive grasstree leaf mass at the time of first measurement (mean inflorescence length was 0.66 m). The larger size of grasstrees at this site in banksia woodland (compare MSB98 grasstrees with YSB98 in Table 3.1) may hold the key to understanding this difference. Size-dependent effects of reproduction have been described previously (Shipley & Dion 1992, Greene & Johnson 1994, Saulnier & Reekie 1995). Saulnier and Reekie (1995) recorded negative effects of reproduction on growth of young plants of *Oenothera biennis*, while larger plants with greater nutrient reserves were unaffected.

Size is often a consequence of resource availability. The proportion of carbon required to support early inflorescence growth relative to the size of the available starch stores may account for a negligible effect on early leaf mass in the large grasstrees in banksia woodland. Supporting my interpretation, the results from this study indicate that, compared with the banksia woodland grasstrees, a larger proportion of leaf growth was sacrificed by the jarrah forest grasstrees (7% more, although not significantly different) to produce a shorter final inflorescence (about 50% shorter). In keeping with these observations, *X. preissii* inflorescence size and fruit number on the Swan Coastal Plain are considered largely proportional to the size of the plant (Meagher 1974). It is likely that *X. preissii* inflorescence size is a balance between the advantages associated with pollinator attraction and the increased cost of reproduction. Assuming this relationship, the two-fold difference in mean mature inflorescence length between jarrah forest and banksia woodland might reflect constraints imposed by differences in resource availability between the two habitats. For example, light availability is greater in banksia woodland (Chapter 5), and larger grasstree stem circumferences (Chapter 3) also imply greater general resource availability to *X. preissii* in the same habitat.

Almost exclusively triggered by the passage of fire, the start of the reproductive season for *X. preissii* is still strongly governed by seasonal changes from winter to spring. While late spring temperatures are more likely to promote rapid inflorescence growth, an early start in August/September is necessary to ensure that seeds can be set before winter soil moisture reserves are depleted during early-summer. Given this interpretation, the urgency to commence reproduction is likely to be an adaptive response to water availability. The water content of the inflorescence during its development and maintenance, up until pollination, was 80%, which is 10% higher than leaf water content (D. Korczynskyj, unpublished). During anthesis, sticky nectar was exuded from the spike attracting an abundance of invertebrates, and the removal of desmium tissue cores while sampling for starch from reproductive grasstrees induced a trickle of syrupy solution laden with sugar, while samples from vegetative plants remained dry. Despite these characteristics, predawn Ψ_X measured in both jarrah forest and banksia woodland did not indicate any extreme or even additional water stress on reproductive grasstrees, as a consequence of increased water demand by the inflorescence. In fact, less negative midday Ψ_X suggested that the foliage and inflorescence, comprising the crown of the reproductive grasstrees, transpired less water than the vegetative grasstrees with foliage only. This somewhat puzzling result may be attributed to a reduction in foliage surface area of the reproductive grasstrees resulting from less leaf production, and so compensating for the relatively high water demand of the inflorescence compared with the leaves.

Trends in the water status of reproductive and vegetative grasstrees over time showed consistency between the jarrah forest and banksia woodland sites, although predawn and midday Ψ_X were generally lower in the jarrah forest. Such divergence between grasstrees of the two sites points towards differences in habitat water availability, and is an example of a possible resource difference that would contribute to the disparity in inflorescence size described earlier.

Some of the carbohydrates synthesised by the foliage of *X. preissii* are converted into insoluble starch grains in the desmium. Despite the narrowness of this tissue layer (< 5 mm; Fig. 4.3), for a waist height grasstree, total starch reserves are estimated to approach 0.5 kg, approximately half the weight of a mature inflorescence at anthesis (Fig. 4.14). During reproduction this starch is remobilised in the form of soluble

carbohydrates and redistributed to the developing inflorescence. There it is used for early inflorescence growth, and fruit and seed production, evidenced by the reduction of desmium starch during these critical periods (Fig. 4.10). Fastest inflorescence elongation occurred during early inflorescence growth (see Fig. 4.5 and Fig. 4.14 ("no cover" treatment)), which is in keeping with other grasstree species (Cleland 1913, Staff 1976). This correlation supports the interpretation that desmium starch is consumed during times of peak growth, whether vegetative (Chapter 3) or reproductive. By the time seeds were released, 60 % of the desmium starch reserve had been consumed (about 0.296 kg), adding to the cost associated with reproduction. In some species such use of stored resources is likely to reduce or completely negate any visible signs of reproductive cost (Horvitz & Schemske 1988). By drawing on reserved energy to support reproduction, *X. preissii* must later suffer the trade-off of reduced growth while restoring the depleted reserve.

Leaf production after seed release continues from the lateral apices (axillary buds) that exist close to the base of the scape during reproduction (Staff 1968, Gill 1993). At this time, leaf production by jarrah forest grasstrees was typical for autumn (0.6–1.0 leaves/d), which superficially implied that no negative effect of reproduction was sustained. Contrary to my prediction that leaf removal following reproduction would totally deplete an already scant starch reserve and result in reduced growth, the observed response suggested that *X. preissii* was adequately equipped to endure the treatment. The only cost of this treatment was visible as a further depletion of the remaining desmium starch reserves. Herbivory of grasstrees by parrots (McNee 1997) and kangaroos (Fig. 5.1) is more likely when the crown is damaged or recovering after fire, offering easier access to the more tender inner leaves through the needle-like leaves. Also, given the high occurrence of parrot-damaged grasstrees during March in southwestern Australia (McNee 1997), the retention of adequate carbon reserves by *X. preissii* following reproduction could represent an important adaptation to cope with post-flowering environmental stress. The same logic applies to the less likely, yet possible (Lamont & Downes 1979), occurrence of a second fire during this dry period.

A brief methodological note is worthy of mention. Spike and seed predation by the larvae of the broadly distributed moth species, *Meyriccia latro,* has been described previously for *X. australis* (Edmonds & Specht 1981, Staff & Waterhouse 1981). The

voracious and gregarious feeding habit of this species on *X. preissii* led to the destruction of entire inflorescences on several occasions. Protection against such an attack by the application of a non-systemic insecticide was only partially successful, as larvae damage required some grasstrees to be replaced. This result suggests that the larvae may have already been present prior to the first spraying. More detailed knowledge of the timing of this species' life history in relation to the reproductive development of its grasstree hosts may help determine a more effective spraying regime.

4.4.1 Experiment 2: the importance of three carbon sources to reproduction

The initial concern that covering treatments used in the second field experiment may result in high leaf and inflorescence temperatures, negatively impacting inflorescence growth and complicating treatment affects, was warranted. Leaf temperature was greater for covered plants, but the maximum mean value only reached about 34 ˚C, just 2 ˚C higher than air temperature corresponding to maximum leaf production. Generally, leaf and inflorescence temperatures were not excessive, consistent with upper photosynthesis optima for a range of species (Devlin & Barker 1971, Mohr & Schopfer 1995, Fukui 2000), and considerably lower than maximum thermal thresholds given for sclerophyllous mediterranean flora (Larcher 2000).

Leaf fluorescence rose then fell across the range of leaves, from the pale young leaves to intermediate to mature leaves — photosynthetic activity increases as chloroplasts enlarge and mature, and then declines, as leaves grow older (Devlin & Barker 1971). Prior to anthesis, the green inflorescence (spike and scape) was capable of the highest level of light absorption (measured as relative fluorescence) recorded for *X. preissii*, demonstrating its capacity for carbon fixation.

From the statistical analysis of the field experiment data it could be concluded that the inflorescence does not significantly support its own growth. However, all three sets of data suggested that in fact the inflorescence does contribute some energy to its own growth. Heilmeier and Whale (1987) demonstrated that for two closely related biennial species the contribution to the total carbon requirement of their flowerheads, by the same structures, was double for *Arctium lappa*, which bore large green bracts. The mean starch index for those grasstrees subjected to the inflorescence covering treatment was

35% lower than for those grasstrees with no covering imposed, indicating that starch was consumed to compensate for the lack of input by the inflorescence. Unfortunately, low replication due to time limits, and lack of suitable grasstree specimens, resulted in high standard errors and subsequent lack of significance.

This surprisingly small contribution of energy from the inflorescence places primary importance on the foliage as the source of most energy during reproduction, followed closely by the desmium starch reserve. It is the combination of these two energy sources and the timing of the reproductive phenophase, enabling the exploitation of optimum water availability and temperature, that allows rapid inflorescence elongation in *X. preissii* and its high reproductive success.

Understanding the relative importance of these three carbon sources to reproduction, it seems likely that grasstrees burnt in autumn which then flower in the following spring would be at a greater disadvantage than those burnt in spring. For grasstrees burnt in autumn, the carbohydrates mobilised from the desmium are used to support resprouting in the spring-summer growing season (Chapter 3). In the event that these plants flower in the first spring following the autumn-burn, the inflorescence becomes an alternative sink to the resprouting foliage for desmium-derived carbon. Bowen and Pate (1993) described a similar use of stored starch by the woody shrub, *Stirlingia latifolia*, in banksia woodland on the Swan Coastal Plain. Particularly for grasstrees burnt in late autumn (May), little time exists to resprout before reproduction commences and immediate daily air temperatures are far from optimal for growth. Therefore, while the incomplete crown remains small, and starch reserves already partially depleted (Fig. 3.13), reproduction commences, postponing further leaf production and continuing starch consumption. This sequence of events, within the relatively short time frame, not only describes a very energy demanding set of circumstances for *X. preissii*, but also possibly represents the plants least-optimum capacity to support inflorescence growth and then developing fruits.

As a consequence to reproductive success of the above scenario, inflorescence mass was 33% lower for the late autumn-burnt grasstrees, primarily from reduced spike mass. As water and nutrients would have been at their peak in their respective cycles (ie. annual and inter-fire) this undoubtedly suggests that carbon was limiting for these plants. But

whether an equivalent reduction in seed number, and therefore reproductive success, was experienced is mere conjecture. However, Lamont et al. (2000) showed, for *X. preissii,* that fruit density per spike in autumn-burnt plants was 80% of that of spring-summer-burnt plants, supporting my interpretation. For the conservation of *X. preissii*, management considerations should include the reproductive costs imposed by the timing of fire, and prescribed burning during spring or early autumn should be selected preferentially.

CHAPTER 5

Environmental causes of grasstree recovery after fire, and the carbon cost of herbivory: investigation through two simulation experiments

5.1 Introduction

Many authors have speculated about the cause of the rejuvenating effect that fire can have on subsequent adult growth (Naveh 1967, Christensen *et al.* 1981, Rogers & Westman 1981, Zammit 1988. Also see my Discussion in Chapter 3). The causes that have been investigated vary substantially. The factors most frequently shown to affect postfire vegetative vigour include: nutrient enrichment of the soil (Simms 1985, Hulbert 1988, Zammit 1988, Witkowski *et al.* 1990, Grogan *et al.* 2000, Kennard & Gholz 2001); changes in water availability (Specht 1957b, Fleck *et al.* 1995); decreased level of shading (Vila 1997, Holmgren *et al.* 2000); increased soil temperature (de Groot & Wein 1999); or a combination of these elements (Daubenmire 1968, Stoneman *et al.* 1995, Gentle & Duggin 1997b). Additionally, the destruction of phytotoxic and antibiotic agents in litter and soil (Naveh 1974, Christensen & Muller 1975, Gentle & Duggin 1997a), changes to bacterial and mycorrhizal fungal populations (Christensen & Muller 1975, Dhillion *et al.* 1988, Bellgard *et al.* 1994, Wilson *et al.* 2001), and the idiosyncratic effects of residual charcoal (Wardle *et al.* 1998), have all been associated with changes in growth after fire.

The predominant finding that improvements in water, nutrients and light availability are probable causes of extraordinary postfire growth is not surprising, considering that these elements represent fundamental requirements for production of organic compounds for growth (Neales 1994). While the importance of water, and to a lesser extent light (daylength), have been explored in previous chapters in relation to leaf production in *X. preissii*, the role of nutrients in limiting grasstree growth has not received the same attention.

Ash produced from burning natural vegetation is nutrient-enriched, with high concentrations of essential elements, including phosphorus, magnesium, potassium and

calcium (Hatch 1960, Raison *et al.* 1985a). Concentrations in ash of these elements can vary according to fire intensity (Stark 1979, Kennard & Gholz 2001), especially phosphorus (Romanyà *et al.* 1994), which can be particularly high in fine, grey or white (mineral) ash (Raison *et al.* 1985a, Raison *et al.* 1985b). Nitrogen, the most important element for plant growth, is readily volatilised during fire and lost in relatively large quantities (Raison *et al.* 1985a, Raison *et al.* 1985b). However, work has shown that fire can commonly cause an increase in soil ammonium levels, resulting in high rates of primary production in re-establishing plant communities (Grogan *et al.* 2000). While a portion of all nutrients is lost during fire through their volatilisation or as particulate matter in the smoke column (Raison *et al.* 1985a), the redistribution of surface ash by wind and water is considered an important factor in the dynamics of the ecosystem (Grogan *et al.* 2000). Not surprisingly, the most significant effect of ash and partially-burnt material on soil nutrient concentrations is detectable in the surface soil horizons (Grove *et al.* 1986, Adams *et al.* 1994, Romanyà *et al.* 1994) and decreases rapidly with depth (Hatch 1960).

The gradual leaching of nutrients from ash over time means that ash acts as a slow-release plant fertiliser under natural conditions (Stark 1979). After one-year postfire, soil nutrients levels return to those similar to prefire concentrations (Grove *et al.* 1986). For a plant community recovering after fire, this suggests that resprouting components are likely to benefit the most from this limited period of enhanced nutrient availability, absorbing nutrients immediately via their mature root systems, in contrast to those species regenerating from seeds later on (Biswell 1974, Keeley & Zedler 1978, Grove *et al.* 1986). However, other evidence suggests that the nutrients released by fire are inaccessible to new growth of resprouting plants because of the superficial penetration of these nutrients into the soil (Wisheu *et al.* 2000). Changes in postfire leafbase concentrations of nitrogen, calcium, potassium, and zinc in *X. preissii* indicate an increased availability of nutrients to this species following fire (Burrows 1998, Ward *et al.* 2001), and may explain the rapid postfire recovery of the crown (Chapter 3). Increases in plant nutrient concentrations and leaf/shoot growth after fire are consistent with the response of various species to nutrient additions in the form of fertiliser (Christensen & Muller 1975, Hulbert 1988, Witkowski *et al.* 1990). Results from observation of plant and soil water status following fire vary considerably, representing both positive (Specht 1957b, Stoneman & Schofield 1989, Stoneman *et al.* 1995),

negative (Christensen & Muller 1975, Sakalaukas *et al.* 2001) and less commonly neutral (see Daubenmire 1968 for review) consequences to plant growth. Most of this research focuses on the effect of the removal of vegetation cover by fire, suggesting either a reduction in transpirational water loss, or an increase in incident radiation and subsequent evaporational loss. Further subtleties have been suggested in regards to changes in surface albedo (Christensen & Muller 1975). Consistent with the effect of burning on the water status of *X. preissii* (see Section 3.3.3) are the results presented by Fleck *et al.* (1995), who suggest that reduced leaf area of two Spanish shrubs following wildfire resulted in improved midday water status. Also, given the tightly controlled, yet continuous, water cycling over summer and fast response to summer rainfall of *X. preissii* (Crombie 1992, and Section 2.3.3.2), it seems likely that this species would readily respond to any improvement in water status following fire.

Additional to the direct effect of flame contact, heat transferred from a fire via convection and radiation can act to significantly reduce overstorey foliage density, effectively opening the canopy. A low intensity fire (300 kW m^{-1}) in jarrah litter on a cool spring day can generate sufficient convected heat to scorch the crown up to 8–10 m above the ground, and possibly up to 20 m during warm, dry conditions in summer or early autumn (Bell *et al.* 1989). As fire-scorched branches are usually killed (Gill 1978), such an event would cause the loss of all scorched leaves and general thinning of the canopy, allowing greater light penetration. Given that jarrah (*Eucalyptus marginata*) can grow to 60 m, the lower overstorey height of banksia woodland, dominated by *Banksia* spp., would be more susceptible to total canopy defoliation, at similar fire intensities. Studies using artificial shading of resprouting vegetation to simulate shoot competition for light after fire between neighbouring species have unambiguously revealed a negative effect of shade on growth (Hulbert 1988, Gentle & Duggin 1997b, Vila 1997). It therefore seems likely that, regardless of habitat, the defoliation of the overtopping canopy by fire may result in a positive effect on subsequent grasstree growth.

Many studies evaluating possible causes of rapid postfire plant growth could be criticised for seeking single causes rather than the likely combination of factors (Hulbert 1988). Even though grasstrees represent a group of plants that are far from subtle in their response to fire, identifying the key postfire elements likely to promote their

characteristically vigorous regeneration poses a difficult problem. In this chapter the following hypothesis was investigated through a fire simulation study.

- Increased water and ash-derived nutrients, and decreased canopy shade promote vigorous leaf growth of *X. preissii* after fire.

Until now, discussion of grasstree growth in my thesis has concentrated on the abiotic factors (particularly fire) that stimulate the most marked responses from *X. preissii*. Herbivory is a biotic interaction identified as important to the survival of *X. preissii* (McNee 1997), and to an extent can induce similar responses in grasstrees as those caused by fire. *X. preissii* has a considerable capacity to regenerate its crown following pruning by Port Lincoln Ringneck parrots (*Barnardius zonarius semitorquatus*) (McNee 1997) similar to vigorous regrowth after fire. In other species, morphological responses (eg. increase branching frequency, increased shoot dry weight and reduction of long-shoots) (Bergström & Danell 1987), changes in nutrient allocation (Danell & Bergström 1989) and stem death are common results of defoliation by herbivores and pruning by fire (Bergström *et al.* 2000). Undoubtedly, fire is a dominant environmental factor currently impacting *X. preissii*. However, some lines of speculation suggest that the importance of the roles of fire and herbivory on Australia's vegetation have only relatively recently swapped (Flannery 1994).

Grazing can occur far more frequently than fire, which raises the question of grasstree resilience to such pressure. The foliage represents the most accessible, palatable portion of a grasstree, and is readily eaten by domestic sheep and cattle, cockatoos, parrots, kangaroos and possibly brush wallaby and rabbits (Halford *et al.* 1984, Long 1984, McNee 1997, Richardson *et al.* 2000). Access to the crown by large animals is impeded by the orientation of the needle-like leaves projecting from the centre towards sensitive areas around the muzzle and eyes. For birds, the crown structure does not offer suitable perching sites and as a consequence damaged grasstrees are targeted for ease of penetration to the more palatable centrally-located young leaves (McNee 1997). Fire markedly increases crown exposure to grazing by herbivores (Fig. 5.1), reducing leaf length and removing the sharp protective tips. Once a damaged grasstree is located it commonly receives more frequent visits than other grasstrees in the area (McNee 1997), establishing a routine of ongoing damage. The combination of fire and grazing often has

a greater impact on a species than the sum of their individual effects (see Whelan 1995 for a brief review). On Rottnest Island, off the coast of south-western Australia, the combination of frequent fires and herbivory by a small macropod, the quokka (*Setonix brachyurus*), was suggested to have caused the demise of the fire-resistant yet highly palatable species *Acacia rostellifera*. The intense grazing pressure applied to resprouting *Acacia rostellifera* after fire forced the gradual dominance of the less palatable *Acanthocarpus-Stipa* low dense heath (Pen & Green 1983).

Fig. 5.1 A short (< 60 cm) grasstree subjected to repeated kangaroo grazing of its apex since being burnt in spring 1998. An equivalent-sized, ungrazed grasstree can be seen in the background.

Grazing can induce changes in plant nutrient acquisition, the redistribution of photosynthates, mobilisation of stored reserves, increase in photosynthetic capacity, reduction of tissue senescence, and can even increase growth vigour stimulated by thiamine from herbivore saliva (see McNaughton 1983 and Lindroth 1989 for reviews, and Kosola *et al*. 2001). These changes can directly affect the performance of recovering plants, causing undercompensation, exact compensation or overcompensation of regrowth, relative to the growth rate of unaffected plants (Crawley 1983). The few studies that have revealed overcompensation in shoot biomass (Edenius *et al.* 1993), due

to enhanced plant performance after herbivory, have most often been short-term, and the responses of these plants may differ after successive years of defoliation (Lindroth 1989). Some authors have generalised that this response is more likely among those species growing on nutrient-rich soil, whereas in nutritionally-poor environments more energy is expended in chemical defence (Lindroth 1989).

When a substantial area of green leaf remains after grazing, starch reserves are relatively unimportant for regrowth (Crawley 1983), as newly synthesised photosynthates and existing soluble carbohydrates are sufficient to meet the respiratory needs of the surviving tissues and support compensatory growth (Kigel 1980, Kosola *et al.* 2001). However, when herbivore damage significantly reduces photosynthetic surface-area, reserve starch can be crucial to plant survival (Donart & Cook 1970, Bowen & Pate 1993), and is remobilised to fill the role of the rapidly consumed residual soluble carbohydrates. The dependence on carbon reserves holds important implications for the survival of plant populations vulnerable to chronic herbivory. Repeated shoot removal has been shown to exhaust starch reserves and retard regrowth or even cause death in a range of tree and shrub species (Bamber & Humphreys 1965, Miyanishi & Kellman 1986, Bowen & Pate 1993). Slow growing species that are able to withstand chronic herbivory may still suffer from other detrimental effects, such as reduced fitness. Slow growth of Scots pine (*Pinus sylvestris*) saplings in Spain disposes these plants to browsing of their leader terminal shoots by livestock and wild ungulates for many years, retarding their development to maturity (Zamora *et al.* 2001). Although *X. preissii* is never able to escape bird herbivory, its slow vertical growth disposes it to grazing from domestic stock and kangaroos for potentially over 100 years (see Lamont & Downes 1979 for vertical growth rate), after which they may attain a height out of reach of these animals.

Specific to the herbivory of *X. preissii* by parrots is the detrimental effect of the residual pieces of leaf left behind, suggested to rot the apical meristem (McNee 1997) and potentially reduce early initiation of active export of photosynthate from young leaves (Mattheis *et al.* 1976). The combination of three years of parrot browsing (5–6 months per year) and the smothering effect of the rotting chaff left by the foraging parrots may result in the death of the crown (McNee 1997). From these observations it can only be speculated why these grasstrees ultimately die. Did the frequency of herbivory exhaust

dwindling starch reserves preventing the grasstrees from continued regeneration, or was apical rotting wholly responsible? From these questions and my accumulated knowledge of grasstree response to disturbance the following two hypotheses were generated, which were the focus of the simulated herbivory study described in this chapter (see earlier for the hypothesis pertaining to the fire simulation experiment).

1) Grasstrees leaf and biomass production is stimulated by low frequency herbivory, but is reduced by frequent herbivory.
2) The depletion of grasstree desmium stores is positively correlated with the frequency of herbivory pressure.

Lastly, I comment on the decision to undertake field-based experiments. For manipulative experimentation, the use of seedlings in glasshouse pot trials or in pseudo-natural field trials are a popular means of simplifying a complex system in order to observe the effect of a manageable number of variables. However, the simpler the simulation and the more complex the simulated system, the less representative the results are of the natural circumstances under investigation. For this reason, generalisations from such research can be criticised (Roger Cousens, plant ecologist, University of Melbourne, pers. comm.). Size and morphological differences between mature plants and seedlings of *X. preissii*, suggests that there would be little value in drawing conclusions of established populations from seedling trials. Additionally, the subterranean existence of the seedling apical meristem (Koch & Bell 1980, Staff & Waterhouse 1981, Gill 1993) acts to widen the gap between the likely response of seedlings and mature grasstrees to fire and herbivory. Therefore, as the logistics of performing fire and herbivory simulation experiments under controlled conditions using mature plants are physically unachievable, the decision to undertake field-based studies using naturally occurring mature grasstrees was made. Although this experimental strategy undoubtedly introduced a higher level of inherent variability to the results, it also more closely approximated the natural situation.

5.2 Materials and methods

5.2.1 Experiment field sites

The manipulative field experiments described in this section were undertaken at long unburnt sites in jarrah forest or banksia woodland. The fire simulation experiment was carried out at the single field site YFSE (Table 1.1), directly opposite the spring-burnt YSB98 banksia woodland sites, yet separated by a 6 m wide, sand track (used as a fire break during the 1998 fire). This site has a northerly aspect with a gentle slope, typical of the dune system to which the area belongs. The simulated herbivory experiment was replicated at both jarrah forest (MC; Table 1.1) and banksia woodland (YC) sites.

5.2.1.1 Experiment 1: Simulating three environmental factors considered to characterise postfire banksia woodland

This experiment involved the manipulation of water availability, ash derived nutrients and shading, which were considered important to the growth of resprouting vegetation following fire. A factorial combination of the three factors at two levels (presence and absence) allowed eight possible treatments to be randomly applied to 48 grasstrees between the heights of 0.8 and 1.3 m (Table 5.1), giving six replicates per treatment. Twelve additional grasstrees were allocated to one of two extra intermediate shading treatments (50% and 70% shade), complementing the two extreme levels of shading used in the main experiment (0% and 90% shade). Shading was the only treatment imposed on these grasstrees, to enhance the analysis of the effect of shading on *X. preissii*. In addition to height, circumference was recorded for all grasstrees (0.4 m from the ground), and consistency of plant girth between treatments was checked using a one-way ANOVA to control this possible source of variability (see Section 3.2.2.1).

The commencement of the experiment on 10 June 1999, although potentially 1–2 weeks late, was representative of the likely timing of a late-autumn prescribed burn. However, shade was the only treatment imposed at this point. Watering commenced prior to summer on the 26 November 1999, coincident with the start of the growing season (see Chapter 2). Nutrients (i.e. ash) were applied between 1 July and 20 July 1999, synchronised with the first substantial winter rainfall for 1999, preventing premature

loss of ash by wind. Figure 5.2 shows the fire simulation site at the commencement of the experiment.

Table 5.1 Description of the 10 treatments applied to grasstrees during the fire simulation experiment. The first eight treatments comprise a combination of the three factors, water, ash and shade, and the last two treatments were added to provide extra detail of the effect of shading. Six grasstrees were assigned to each treatment.

Treatment	Description
Control	No shade, water or ash applied
+water (–ash, –shade)	Water provided only
+ash (–water, –shade)	Ash provided only
+shade (–water, –ash)	90% shade provided only
+water, +ash (–shade)	Water and ash provided
+water, +shade (–ash)	Water and 90% shade provided
+ash, +shade (–water)	Ash and 90% shade provided
+water, +ash, +shade	All three factors applied
Additional shading treatments:	
50% shade	50% shade provided only
70% shade	70% shade provided only

Fig. 5.2 This overview of the YFSE (Yanchep fire simulation experiment) site was taken from a nearby south-facing hill in the YSB98 site. The two pale green water tanks (side by side) can be seen in the centre and dark green tents can be seen scattered through the landscape from east to west.

Pre-experiment preparation

Prior to the application of treatments each grasstree was totally defoliated (living and dead leaves removed) using two-stroke hedge-cutters to simulate the 'pruning' experienced during a fire (Fig. 5.3a,b). Also, at this time (18 to 25 May) all vegetation

Fig. 5.3 (a) the author using a motorised hedge-cutter to remove all dead (skirt) and living leaves, simulating the loss of foliage associated with fire. (b) the same grasstree after the commencement of the experiment (regrowth visible at the apex) can be compared with an intact grasstree of similar height in the background. Also, note the ash distributed around the base of the grasstree as part of the treatment +ash, –water, –shade.

within a 4 m radius was cut to ground level using two-stroke brush-cutters, or defoliated in the case of large plants using hand-saws and secateurs. This process was used to reduce the variation in competition for water and nutrients imposed by other species on each grasstree. Defoliation was repeated on three other occasions (24 September 1999, 1 March 2000 and 17 March 2000). All cut foliage during defoliation and clearing was raked beyond the 4 m radius (Fig. 5.4a,b).

Fig. 5.4 (a) adjacent grasstrees after being pruned, but still surrounded by clipped foliage and ground cover. (b) a group of grasstrees one week after the commencement of the experiment with all clippings and ground cover removed (shading treatments applied).

Factors comprising each treatment

Water

Water was supplied from two 9,000 L fibreglass tanks placed uphill of the site (Fig. 5.2) and filled with a combination of ground and Wanneroo scheme water using heavy-duty fire trucks, courtesy of the Department of Conservation and Land Management. All trucks used were thoroughly rinsed before being filled to ensure the removal of residual fire retardants. A sample of water from each tank was analysed for trace elements (Marine and Freshwater Research Laboratory, Murdoch University), pH (1228 pH meter, Beckman Instruments, Germany) and conductivity (HI 8820N conductivity meter, Hanna Instruments, Portugal) and compared with rainwater collected at the site. A gravity-fed hose (19 mm) system ran water from the tanks to each pair of 46 L rubbish bins positioned beside each water-treated plant (Fig. 5.5). The flow capacity of this system was later enhanced using a 50 cc, two-stroke, water pump. Each bin supported two continuously running dripper heads placed equidistant around each plant at a radius of 1.25 m, which were covered by tin cans to reduce immediate evaporation. This

Fig. 5.5 Two grasstrees with water and 90% shade applied, and the nearest grasstree has also been treated with ash. Each rubbish bin holds 46 L of water, replaced every two weeks, and fed two dripper heads covered by tin cans via 6 mm black tubing visible in the foreground.

distance was chosen based on a preliminary investigation that identified it to correspond with the zone of highest fine root density at a depth of 0–0.4 m, and comparable root density to closer distances at a depth of 0.4–0.8 m. The rate of water flow was adjusted

to empty both bins within 24–30 h (3.1–3.8 L/h). This flow rate ensured that debris, ants etc. did not block the drippers and prevent watering. A fortnightly watering regime was maintained until 18 May 2000 (autumn), when soil moisture content near watered and unwatered grasstrees did not differ, due to the start of reliable autumn/winter rains.

Soil moisture was measured to demonstrate an effect of the watering treatment, and also as a guide to when the watering treatment should start and finish. Every 1–6 weeks during the watering period (midway between waterings), starting on the 29 October 1999, a hand-operated soil corer was used to extract soil from a depth of 30–40 cm from adjacent to watered and unwatered grasstrees. Samples were placed in labelled, heat-resistant, oven bags, and weighed before drying at 100 ˚C for 48 h. After recording each sample's dry weight, the weight of the soil moisture was calculated as a percentage of wet soil.

Predawn and midday xylem water potential (Ψ_x) was measured for plants subjected to watering treatments during spring (5 September 1999), summer (10 December 1999) and autumn (4 April 2000). A single leaf was collected from each plant and a pressure chamber was used to determine Ψ_x (as described in Section 2.2.5.1). Results were compared to unclipped intact grasstrees from the banksia woodland control site (YC).

Ash

Some of the cut foliage created during defoliation and clearing (including much dead and fresh *X. preissii* leaf) was transferred to the adjacent site (YSB98) and burnt in a controlled fire for the purpose of creating ash. A once off 30 L (0.03 m^3) quantity of fresh ash was spread by hand within a 1 m radius of each stem (visible in Fig. 5.3b and 5.5).

Shade

Shade was provided by an A-frame style tent (Fig. 5.5) built over 24 of the 48 grasstrees using 90% woven green shade cloth (Coolooroo ®, Melbourne, Australia). The tents were positioned with their shading-surface facing north to maximise the period of shade offered to each plant, and were sufficiently large to avoid significant contact between the plant and the cloth. Only during early morning and late afternoon during summer were the lower leaves exposed to sunlight. All tents were maintained during the year, with storm damage repaired within one week.

To quantify the effect of the three shade cloth densities, photosynthetically active radiation (PAR) was measured on 24 February 2000, for open sun and under each

different shade cloth tent, using a quantum sensor (model LI-185B, Li-cor, Lambda Instruments Corporation, Nebraska, USA). Measurements were made over midday within 40 min, during which the weather was fine, with cloudless sky and temperature of 28 ˚C.

Collection of data

In an attempt to reveal an effect of the watering treatment on grasstree leaf production, specific to the dry season, leaf production was monitored for all plants towards the end of the growing season. Each grasstree was monitored for leaf production from 31 March to 27 April 2000 (see Section 2.2.2 for detail of method), during the driest time of the year.

After one year, all leaves on each plant were harvested (19 to 23 June 2000, Fig. 5.6). Leaves existing prior to defoliation at the start of the experiment (identified by blunt incomplete tips) and that had subsequently elongated were bagged separately from the remaining intact leaves initiated during the experiment. Of the new leaves, the leaf tips were removed and also bagged separately so that the number of new leaves produced during the experiment could be counted. All leaf material was oven dried at 70 ºC over 6 days, then weighed. In the two instances when a grasstree had produced an inflorescence, its dry mass was included as part of its total leaf biomass. In addition, a thin wedge was cut from near the apex of each grasstree, for the purpose of collecting leaf bases for nutrient analysis by a colleague.

Fig. 5.6 Harvesting grasstrees after one year involved removing all foliage that was collected on large pieces of shade cloth laid beneath each plant and packaged in labelled brown paper bags.

5.2.1.2 Estimating canopy cover in grasstree habitat

Canopy density was estimated once for an unburnt site in the jarrah forest (MC) and once before and 10 times after fire for a banksia woodland site burnt in autumn, on 22 April 1999 (YAB99) 6 weeks earlier than the start of the fire simulation experiment. Canopy density was measured using a spherical forest densiometer (Ben Meadows Co., Atlanta, USA. Lemmon 1956, Lemmon 1957). Estimates were derived from a series of random measurements: six at the jarrah forest site, and 8–10 at the autumn-burnt site. Each measurement was the mean of four repeat measurements made facing in the direction of the four compass points. Due to the patchy nature of *Banksia* spp. that dominate the canopy of the woodland, measurements in this habitat were only made within those areas where the overstory species existed. At the burnt site, canopy density was estimated once prior to the autumn-burn, then every 6–24 weeks, with the last measurement made two years after the fire.

5.2.2 Experiment 2: Effect of simulated herbivory on the resprouting performance and starch reserves of *X. preissii*

This experiment assessed the effect of five frequencies of simulated leaf herbivory on the ability of *X. preissii* to resprout, and a sixth treatment introducing the effect of residual leaf clippings, often left by parrots after feeding on grasstrees (McNee 1997. Fig. 5.7a,b). At both sites 24 grasstrees were randomly allocated to one of the six treatments listed below:

Control — no herbivory,
Once off clipping (herbivore attack),
Clipping every four months,
Clipping every two months,
Clipping every month, and
Clipping every month, plus clippings applied (Fig. 5.7b).

Because of the potential effect of grasstree size on growth, grasstrees of similar circumference were selected. The removal of all living foliage using secateurs and a hand saw simulated an herbivory event, and small 1–3 cm length pieces of grasstree leaf collected from other grasstrees (not used in the experiment) were placed over the apex to simulate the clippings discarded by parrots (Fig. 5.7b). No living leaves were clipped

from the four control grasstrees, but the inner 20 young leaves were initially tagged with coloured markers (Section 2.2.2) to indicate the youngest leaves present at the start of the experiment. To estimate the initial living crown biomass of the control plants, the leaf material excised from each grasstree was weighed after drying at 70 °C for 72 h, then used to determined the mean grasstree crown dry weight (n = 20). All plants had a 10 cm wide band of dead thatch immediately adjacent to the living foliage removed to help identify those leaves that died during the experiment. Treatments were first imposed on 1 July 1999 for the jarrah forest grasstrees and on 15 July 1999 for the banksia woodland grasstrees.

Fig. 5.7 (a) accumulated small pieces of leaf (10 mm long) left behind by parrots and cockatoos after browsing on the crown of *X. preissii*. (b) *X. preissii* studied in the herbivory experiment after defoliation. Pieces of broken grasstree leaf were applied to simulate the effect described for (a). Note the growth of the young leaves up through the residual leaf pieces.

Wire cages were placed over the crown of all clipped grasstrees to prevent natural kangaroo and parrot damage. The wire cages were identical to those described in Section 4.2.4, and their attenuation of photosynthetically active radiation (PAR) striking the foliage was evaluated in Section 4.3.6.

On each repeat visit, determined by the frequency of each treatment, resprouting foliage was removed, collected in paper bags, oven dried at 70 °C, weighed, and then all leaves

with unclipped tips (i.e. new leaves produced since the last clipping) counted. Also, a count of the number of new leaves produced on the control plants was made, and an additional 10–coloured markers applied. After 16 months (November 2000) all grasstrees including the controls were harvested for the final time. Dry biomass was determined using the method described earlier for all living foliage and also for foliage that had died during the experiment on the control plants. Mean total crown biomass calculated at the start of the experiment was subtracted from the biomass results for the control plants, to estimate the biomass accumulated over the 16–month period. The number of new leaves was also counted.

On completion of the experiment the impact of the different clipping (herbivory) regimes on grasstree starch reserves was assessed. Desmium tissue was sampled from each grasstree (9 November for jarrah forest and 11 November for banksia woodland), from which the starch index was determined (a relative measure of the status of the starch reserves), using the method described in Section 3.2.5.1.

5.2.3 Statistical analyses

All statistical tests were performed using SPSS 10.0 software program (SPSS Inc., Chicago, U.S.A.), and each analysis conducted is indicated with the corresponding results. Levene's test was employed to test for homogeneity of variances for all data, which were either square root or $\log_{10}$ transformed as required and then re-analysed if necessary. Where transformation did not improve sample variance an equivalent non-parametric test was employed. Variability about the mean is quantified as standard error throughout this chapter.

The 0.05 alpha (95% confidence) level is broadly accepted by biologists when looking to detect true departures from a null hypothesis. However, this conventional, yet arbitrary, probability of Type I error (the rejection of a null hypothesis when it is in fact true) should not be considered to be written in stone (Underwood 1997). The power of an experiment (determined by sample size and population variability) needs to be considered, and recognition of the desirability to avoid Type II errors (accepting a null hypothesis when it is in fact false) is important. For biological work, the consequences of applying findings based on an incorrect conclusion, as the result of focussing on

avoiding Type I errors, could be serious (Lamont 1995b, Underwood 1997). For the purposes of the two relatively coarse field experiments described in this chapter, the use of the conventional $\alpha = 0.05$ was reviewed. From my knowledge of the intrinsic variation associated with *X. preissii* leaf growth, the level of replication used in these studies (imposed by restrictions on time and resource allocation) was relatively low. From this, the term coarse, as used here, relates to the likely low power of each experiment, and the associated high probability of Type II error. As a result, considering the likelihood of both Type I and II errors, $\alpha = 0.1$ was considered reasonable from which to draw statistical conclusions regarding the two experiments. This was further justified by that fact that my working hypotheses for the main effects were directional, making one-tailed tests appropriate, whereas ANOVA is a two-tailed test.

For experiment 2, one-way ANOVA was used to initially analyse the effect of herbivory treatments on leaf production, biomass production and stored-starch use (starch index) by *X. preissii*. As the experimental strategy involved the quantification of grasstree responses to a series of levels of herbivory, it was decided not to use a conventional multiple comparison test, which tends to force data into distinct categories. Rather, normal order plots (Perry 1986, Eccles *et al.* 2001), which use a continuous scale, were employed to search for patterns among mean grasstree responses to each treatment, avoiding concealing useful detail. The appropriate use of normal mean plots as an alternative to multiple comparison tests has been demonstrated previously (Perry 1986, Cousens 1988). Levene's test for homogeneity of variance was applied to all data and transformed values were plotted where necessary. All values cited in the text were untransformed where necessary.

5.3 Results

5.3.1 Experiment 1: Simulating three environmental factors considered to characterise postfire banksia woodland

5.3.1.1 Confirming treatments effects

Plant size

To prevent a biased effect of plant size on biomass or leaf production, the circumference of grasstrees from each treatment was compared. No significant difference in circumference size was noted between grasstrees from each treatment ($P = 0.4452$; one-way ANOVA).

Water

The largest difference between rainwater and tank water was the higher concentration of ammonia in the rainwater (Table 5.2). Phosphate was also higher in rainwater compared with tank water used to irrigate the grasstrees, but other differences in the chemical constituents were considerably less.

Table 5.2 Results of a physical and chemical analysis of water, comparing rain collected at the fire simulation (YFSE) field site, and water stored in two fibreglass tanks used to irrigate grasstrees during the 1999/2000 summer. Data are for a single sample from each source.

	Rainwater	Tank 1 (west)	Tank 2 (east)
Conductivity (μs^{-1} cm^{-1})	34	126	110
pH	6.3	6.6	6.4
$NO_3 + NO_2$ (μg N L^{-1})	228	656	104
Ammonia (μg N L^{-1})	2065	21	29
Phosphate (μg P L^{-1})	367	18	6
Potassium (mg L^{-1})	2	5	3
Calcium (mg L^{-1})	46	44	55

On the three sample dates prior to watering, soil moisture for watered and unwatered treatments showed a similar pattern of decline (Fig.5.8), with the last pre-watering measurements indicating that there was a significant difference between soil moisture of the two contrasting treatments (P = 0.042; two-tailed t-test with sequential Bonferroni correction). One week after the first watering, a treatment effect was demonstrated with a > 2% rise in soil moisture for those plants receiving water that was significant (P = 0.0004). No difference in soil moisture between the watered and unwatered treatments was evident by early April (P = 0.126), and this result was consistent with the last two measurements.

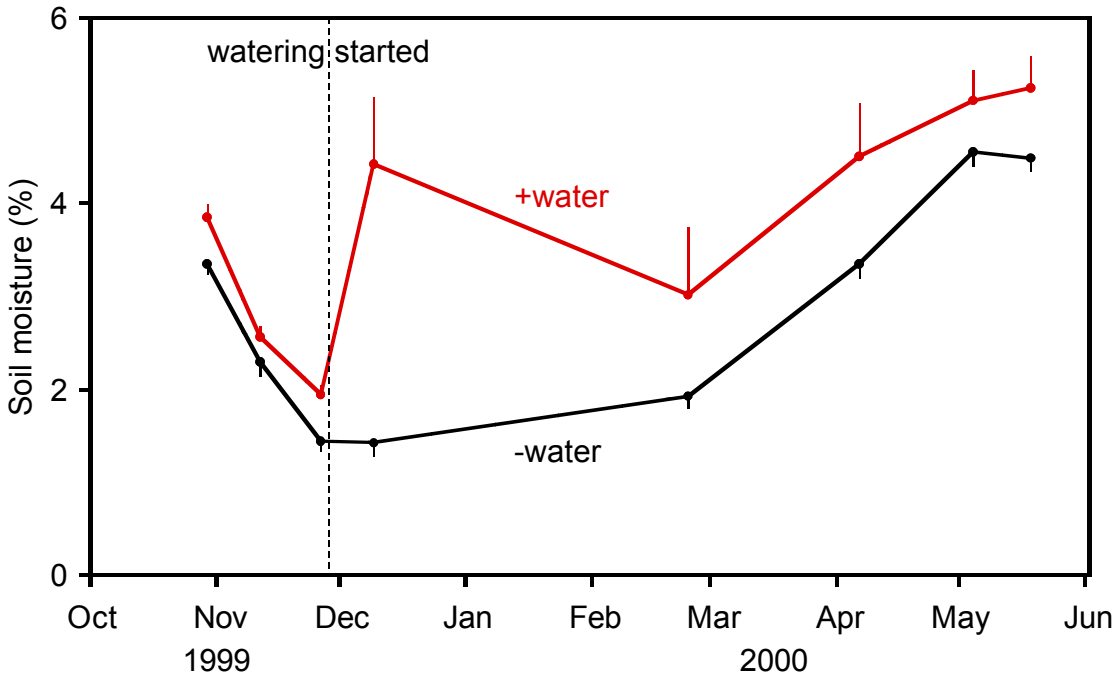

Fig. 5.8 Percent soil moisture of soil collected from beneath those grasstrees irrigated (adjacent to drippers) and those grasstrees without water added, over the 1999/2000 spring-autumn period. The timing of the start of watering is indicated and data are mean of six plants ± standard error.

Seasonal midday and predawn Ψ_x were not very different between watering treatments (Fig. 5.9a,b). Mean summer predawn Ψ_x of watered grasstrees was higher than those unwatered, but the difference was less than 0.3 MPa. Unwatered grasstrees from the fire simulation experiment had consistently lower predawn Ψ_x than the unclipped grasstrees from the control site (YC). While midday Ψ_x of these grasstrees was considerably different during spring, the difference was less pronounced during summer and autumn.

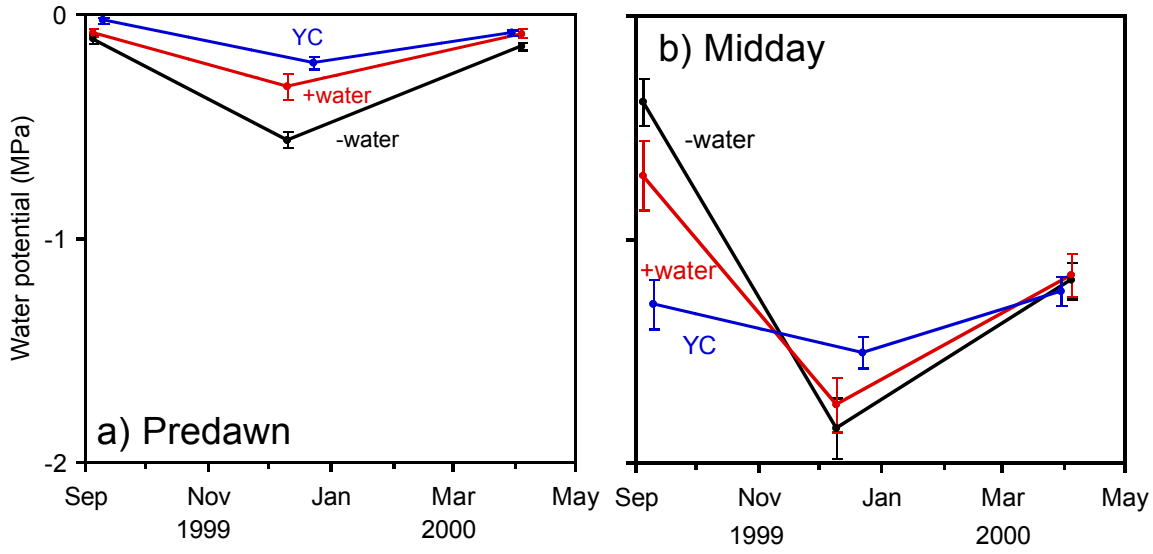

Fig. 5.9 Water potentials measured on watered and unwatered grasstrees during the fire simulation experiment and concurrently on grasstrees from the unburnt, banksia woodland, control site (YC). Measurements were made (a) predawn and (b) midday, once during spring, summer and autumn. Data are mean of six plants ± standard error.

Shade

PAR decreased linearly with increasing shade cloth density (Fig. 5.10).

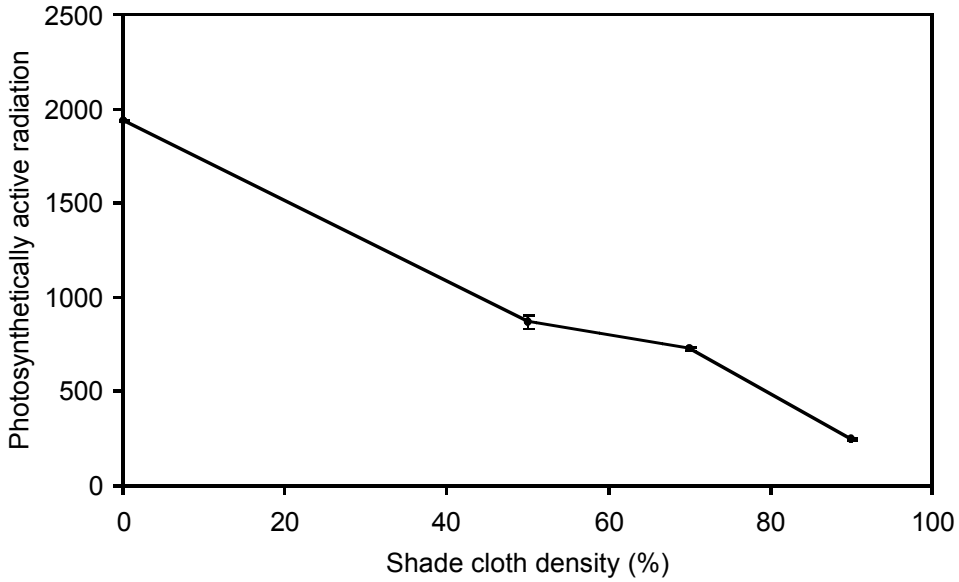

Fig. 5.10 Photosynthetically active radiation (μmol m^{-2} s^{-1}) measured beneath four densities of shade cloth, representing 0%, 50%, 70% and 90% shading. Data are mean of six measurements ± standard error.

5.3.1.2 The effect of water, ash and shade on grasstree biomass and leaf production

Of the three factors (water, ash or shade), ash and shade affected leaf growth (Table 5.3). The addition of ash was associated with greater new leaf biomass ($P = 0.065$, three-way ANOVA), yet played no role in new biomass production by leaves existing at the time of treatment application. 90% shading resulted in a decrease of biomass in both leaf categories as well as in the number of leaves produced. Mean total biomass was 25% greater for grasstrees in full sun and they produced on average 15% more leaves than those under shade cloth. The only significant interaction was between water and shade for pre-existing leaf biomass ($P = 0.090$, three-way ANOVA): when grasstrees were shaded, the addition of water increased biomass, but when in full sun, the reverse occurred.

Table 5.3 Summary of the effect of water, ash and shade on leaf biomass and the number of leaves produced by *X. preissii* during a three-way factorial experiment conducted from 10 June 1999 to 23 June 2000. Data are means of 5–6 plants with SE in parentheses. *P*-values for each of three separate ANOVA analyses are provided.

Treatment factors and level				Biomass (kg)				Number of new leaves	
				Pre-existing leaves		New leaves			
				Shade		Shade		Shade	
				+	−	+	−	+	−
Water	+	Ash	+	1.145 (0.148)	1.100 (0.198)	2.486 (0.284)	2.483 (0.421)	1215 (73)	1489 (143)
			−	0.945 (0.125)	0.985 (0.175)	1.920 (0.131)	2.351 (0.427)	1259 (148)	1230 (83)
	−	Ash	+	0.966 (0.106)	1.231 (0.118)	2.195 (0.188)	2.954 (0.329)	1254 (59)	1427 (65)
			−	0.807 (0.140)	1.281 (0.174)	1.870 (0.281)	2.248 (0.382)	1174 (130)	1384 (145)
Three-way ANOVA (*P*-values)									
Water (W)				0.797		0.976		0.986	
Ash (A)				0.328		0.065		0.352	
Shade (S)				0.094		0.094		0.070	
W × A				0.634		0.717		0.673	
W × S				0.090		0.441		0.762	
A × S				0.497		0.955		0.479	
W × A × S				0.772		0.376		0.231	

There was a minor trend of decreasing biomass and number of leaves produced with increasing shade that was most apparent at the extreme ends of the shading scale (0 and 90%, Fig. 5.11a,b).

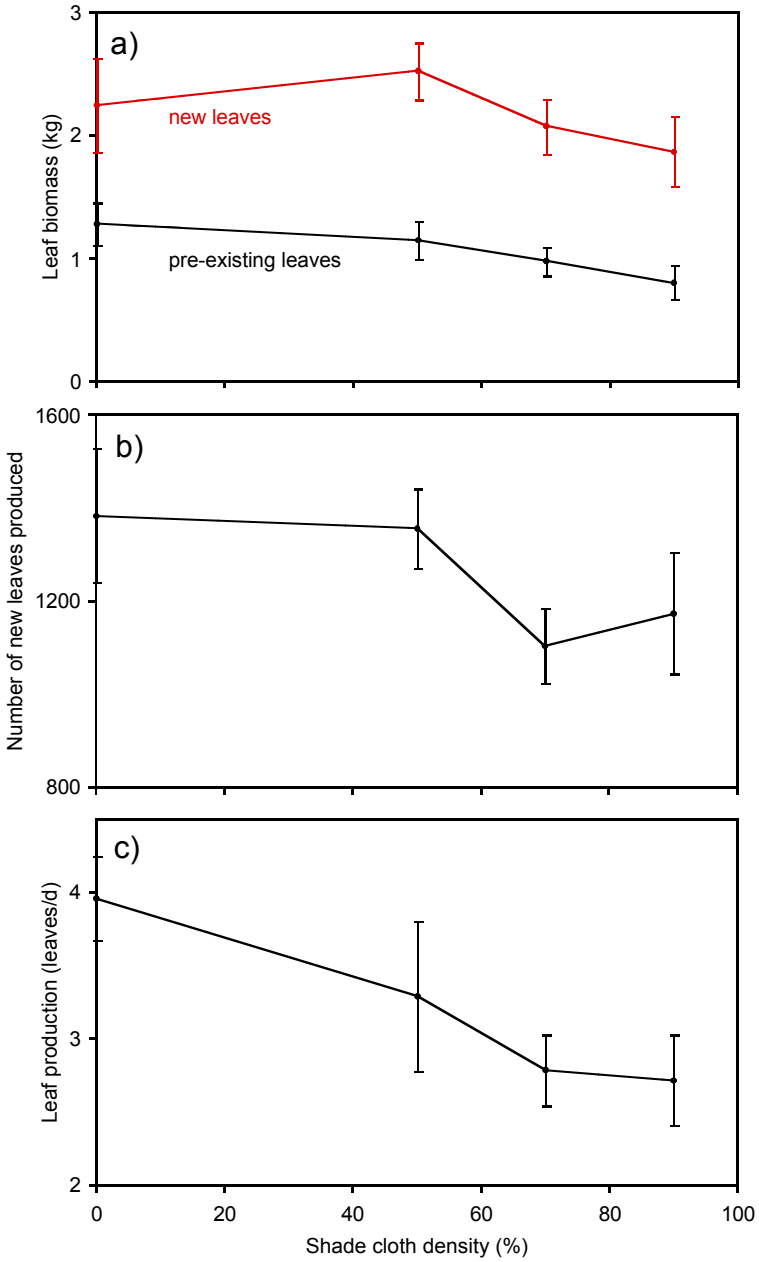

Fig. 5.11 Results for grasstrees after they were defoliated and covered by one of three densities of shade cloth or no cover from 10 June 1999 to 23 June 2000. All data are the mean of six grasstrees ± standard error. (a) dry weight of leaves produced by grasstrees over the period of the experiment, one year. Leaf biomass was separated into pre-existing leaves present at the time of initial leaf removal that subsequently grew back, and those new leaves initiated during the year. (b) the number of new leaves produced by the grasstrees over one year. (c) the rate of leaf production for the grasstrees over a period in autumn from 31 March to 27 April 2000.

Three-way ANOVA of the rate of leaf production during autumn did not reveal any effect of watering (Table 5.4) Consistent with the above results, increasing shade had a negative effect on grasstree leaf production rate during autumn (Fig. 5.11c). Shade had a significant effect on leaf production (Table 5.4), with grasstrees subjected to 90% shade producing leaves at a slower average rate than those in full sun (2.7 ± 0.3 leaves/d compared with 4.0 ± 0.3 leaves/d respectively, Fig. 5.11c). The effect of ash on leaf production was not significant (Table 5.4).

Table 5.4 Summary of the effect of water, ash and shade on the leaf production rate (leaves/d) of *X. preissii* from a three-way factorial experiment, conducted during autumn 2000 from 31 March to 27 April. Data are mean ± SE of 5–6 plants with the results of ANOVA below.

Treatment factor and level				Shade	
				+	–
Water	+	Ash	+	3.06 ± 0.20	3.02 ± 0.64
			–	3.49 ± 0.52	2.80 ± 0.62
	–	Ash	+	2.85 ± 0.17	3.62 ± 0.40
			–	2.72 ± 0.31	3.96 ± 0.29
Three-way ANOVA (*P*-values)					
Water (W)				0.412	
Ash (A)				0.694	
Shade (S)				0.006	
W × A				0.592	
W × S				0.155	
A × S				0.701	
W × A × S				0.634	

5.3.2 Estimating canopy cover in grasstree habitat

Unburnt jarrah forest had a mean canopy cover of 73 ± 1%, considerably denser than the banksia woodland site prior to fire (49 ± 6%). Fire consumed a considerable amount of foliage in the banksia woodland reducing mean canopy cover by 8%, and subsequent leaf drop within seven weeks of fire reduced it by a further 12% (Fig. 5.12). From this point the burnt canopy regenerated most rapidly during the following spring/summer period, surpassing the former canopy density by up to 8% from early February, but returning to pre-fire cover by about two years.

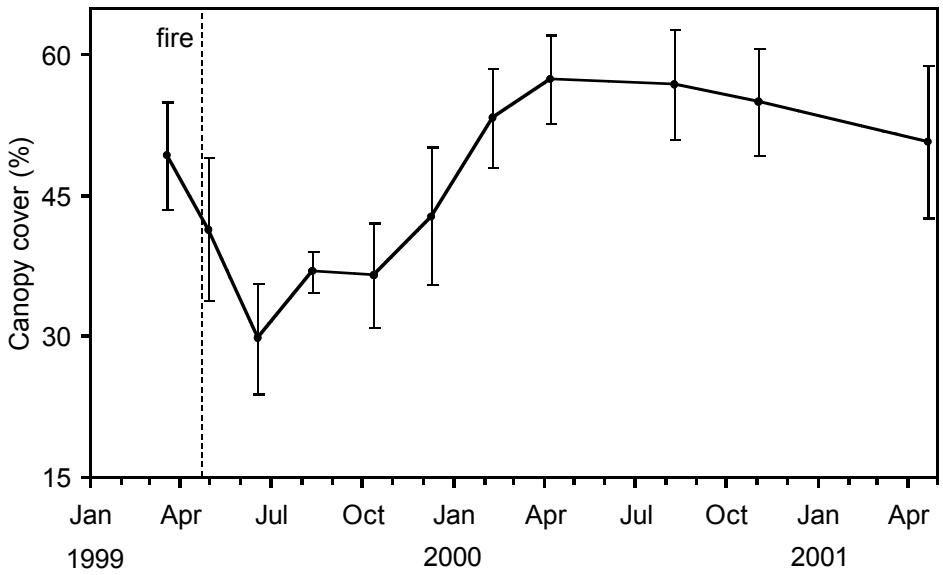

Fig. 5.12 Changes in the percentage canopy density prior to and following an autumn prescribed burn (22 April 1999) in banksia woodland, demonstrating the effect of fire and foliage regrowth. Data are the mean (± standard error) of 8–10 random measurements made using a forest densiometer within an area of canopy cover.

5.3.3 Experiment 2: Effect of herbivory frequency on the resprouting performance and starch reserves of *X. preissii*

Grasstree stem circumference was not different across treatments at either site (P = 0.946, two-way ANOVA), but the larger mean stem circumference of grasstrees used at the banksia woodland site (84.8 ± 1.6 cm) was significantly different from those at the jarrah forest site (70.8 ± 1.3 cm. $P < 0.0001$). There was no interaction between site and treatment circumference (P = 0.911).

Grasstrees from both habitats exposed to simulated herbivory every four months produced the greatest mean number of leaves of any treatment (Fig. 5.13a,b). Control grasstrees at each site contrasted in their mean leaf production performance relative to the other treatments. While those from the jarrah forest showed a relatively large amount of leaf production, the control plants in banksia woodland produced the least number of leaves. Grasstrees for which foliage was removed every two months, every month, as well as being covered in broken leaf, or defoliated only once over 16 months, experienced a similar level of leaf production in both habitats.

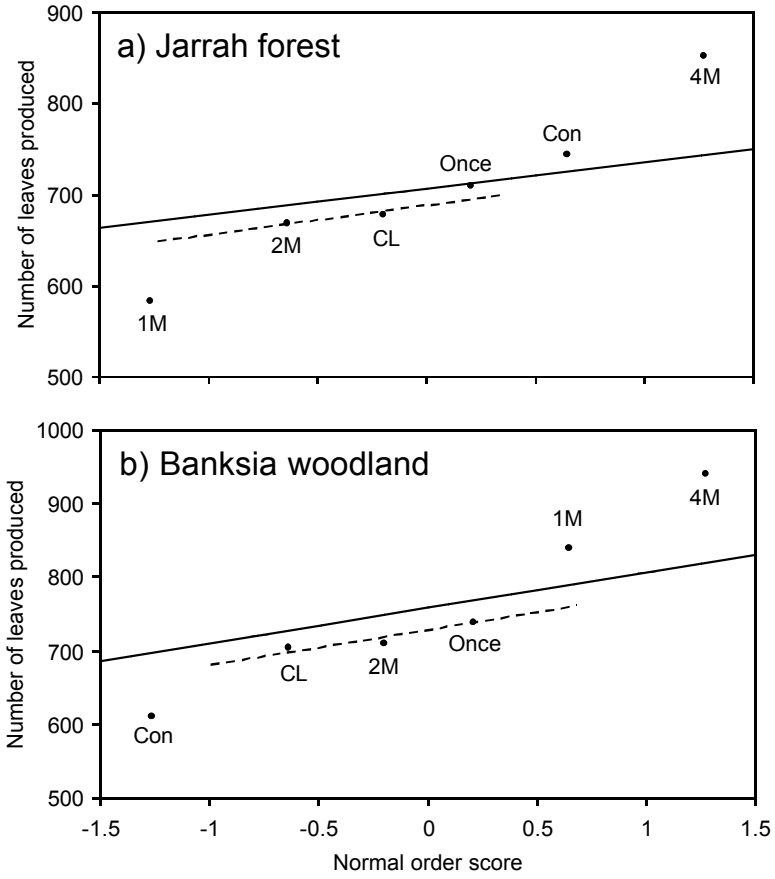

Fig. 5.13 Means (n = 4) of six grasstree treatments of varying clipping frequency and two levels of leaf clippings applied (presence or absence) positioned along the x-axis according to their normal variate scores, and along the y-axis according to the actual values for the number of leaves produced in (a) jarrah forest and (b) banksia woodland, after 16 months. The solid line has a slope equal to the standard error of the mean and intersects the y-axis at the overall mean. Broken lines are simply parallel to this line, and points falling along one of these lines form natural groups that behave similarly. The points are labelled indicating the treatment that they represent; 1M = clipped monthly, 2M = clipped every two months, 4M = clipped every four months, Once = clipped only once at the start of the 16 month period, Con = unclipped control, and CL = clipped every month and had leaf clippings applied to the crown (see Fig. 5.7b).

The relationship between leaf biomass production in jarrah forest grasstrees and clipping frequency was significant ($P < 0.0001$, one-way ANOVA). Leaf biomass produced during the experiment was consistently less for those jarrah forest grasstrees subjected to more frequent clipping (Fig. 5.14a). While not as significant as the jarrah forest results, leaf biomass produced by banksia woodland grasstrees was different across the clipping treatments ($P = 0.065$, one-way ANOVA, Fig. 5.14b). No difference between the effect

of the three highest clipping frequencies (including the treatment where leaf clippings were applied) on leaf biomass production could be distinguished for grasstrees from either habitat (Fig. 5.14a,b). These three treatments caused the lowest level of grasstree biomass production for both habitats, yet the lowest leaf biomass produced by banksia woodland grasstrees (0.805 kg) was over twice that produced by the jarrah forest grasstrees (0.340 kg). The highest mean biomass was produced by the control grasstrees in the jarrah forest (1.423 kg); in contrast, banksia woodland grasstrees responded with leaf biomass production greater than that of the controls (1.081 kg) after a single simulated herbivory event (1.420 kg) and, higher again, after herbivory every four months (1.934 kg, Fig. 5.14b). This effect of the four-monthly clipping treatment on banksia woodland grasstree biomass relative to the other treatments was consistent with the leaf production data.

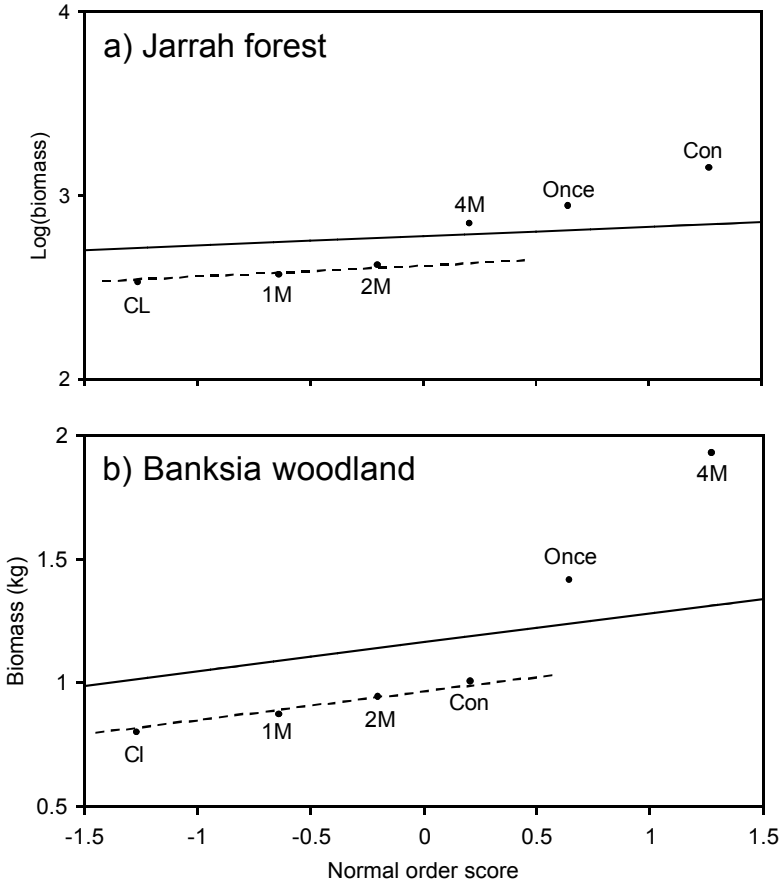

Fig. 5.14 Normal order plots, as described in Figure 5.13 for leaf biomass production response of grasstrees subjected to the same six treatments in (a) jarrah forest and (b) banksia woodland, over 16 months. Each point is the mean of four grasstrees and is labelled as in Figure 5.13. A $\log_{10}$ transformation was used for the jarrah forest data to ensure homogeneity of variances.

The pattern of starch use over 16 months of simulated herbivory, suggested by the relative amount of stored starch present in the desmium (starch index) of each grasstree, was consistent between the two habitats (Fig. 5.15a,b). The starch index was significantly affected by clipping treatments ($P = 0.003$ for jarrah forest grasstrees, $P = 0.011$ for banksia woodland grasstrees, one-way ANOVA), with the four highest clipping frequency treatments resulting in equivalent, low starch indices for those grasstrees. A slight difference between the starch indices of these four treatments was observed for banksia woodland grasstrees (Fig. 5.15b) but this was minor relative to the starch available to control and once-clipped grasstrees (compare the range of mean grasstree starch indices of the four highest frequency treatments = 8.8–11.8, with the mean for the controls = 98.3). In jarrah forest the single clipping treatment was sufficient to reduce grasstree starch reserves below that of the intact control plants, but not in banksia woodland.

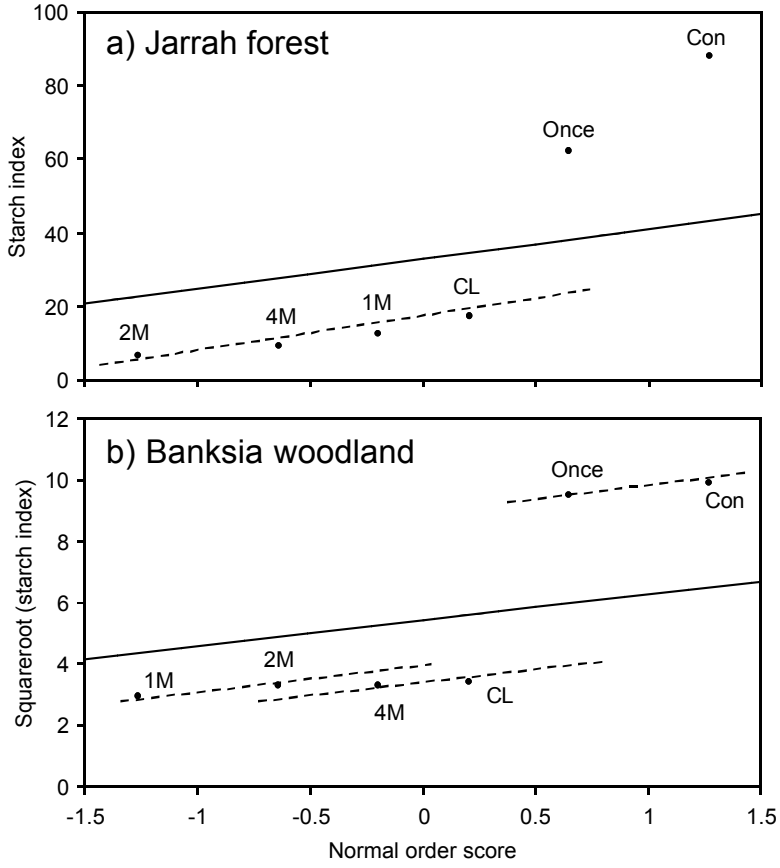

Fig. 5.15 Normal order plots, as described in Figure 5.13, for the relative availability of starch (starch index) in grasstrees subjected to the same six treatments in (a) jarrah forest and (b) banksia woodland, over 16 months. Points are the mean of four grasstrees and are labelled as in Figure 5.13. A squareroot transformation was used for the banksia woodland data to ensure homogeneity of variances.

Finally, the effect of adding leaf clippings to the apex of grasstrees subjected to monthly herbivory was examined. No significant effect of the added leaf material was demonstrated for leaf production (P = 0.390 for jarrah forest, P = 0.334 for banksia woodland, t-test), leaf biomass (P = 0.523 for jarrah forest, P = 0.793 for banksia woodland) and starch index (P = 0.597 for jarrah forest, P = 0.774 for banksia woodland).

5.4 Discussion

5.4.1 Causes of postfire crown vigour

Foliage growth responded differently to each of ash, water and shade, hypothesised as important to grasstree postfire leaf growth. Results indicated that while the presence of ash and reduced interception of PAR after fire can significantly increase biomass and leaf production of *X. preissii*, the characteristically vigorous rate of crown regrowth is unlikely to be supported by an improvement in soil water status. Although the positive effect of reducing shade and adding ash have importance, it is likely that the clipping of grasstree foliage, simulating leaf combustion during fire, was the primary cause of increased grasstree leaf production. Over the year-long experiment, grasstrees subjected to all treatment combinations produced at least twice as many leaves as the number produced by intact grasstrees from the unburnt control site (YC, after making a suitable correction for plant size: see Section 3.3.1.1).

Generally, the mean number of leaves produced by grasstrees in each treatment more closely parallelled the mean number of leaves produced by banksia woodland grasstrees burnt during autumn 1999 (compare 733–989 leaves (range of results across treatments with mean of 854) to 905 leaves). To quantify the effect of clipping, leaf production of grasstrees subjected to the treatment –ash, –water and 50% shade, was compared with leaf production of intact control plants from YC. This selection, and not of the treatment –ash, –water and 0% shade, was based on the assumption that unclipped grasstrees are self-shading, possibly reducing apex temperature and light penetration to the inner leaves, which is compensated for by the 50% shade cloth treatment. The effect of clipping alone can therefore be considered to increase normal grasstree leaf production by 126%, significantly greater than the individual response to any of the three variables explored (compare the range of 1174–1427 leaves produced in response to additions of ash, shade and water with 1356 leaves produced from clipping alone (–ash, –water and 50% shade)).

Ash

The positive affect of ash addition can be attributed to the nutrient enrichment of the soil, universally demonstrated by numerous authors working in floristically disparate environments (Hatch 1960, Stark 1979, Adams *et al.* 1994, Grogan *et al.* 2000). Ash-derived nutrients supported grasstree biomass production, suggesting that grasstree crown development in banksia woodland is limited by nutrient supply in the absence of fire. This is not surprising considering the general acceptance that the shrublands of southwestern Australia, as well as other heath and woodlands throughout Australia, are extremely nutrient impoverished (Specht 1969, Specht 1973, Lamont 1984, Lamont 1995a).

Increased photosynthetic rates, encouraging faster growth of resprouting shrubs in mediterranean Californian and Spain, have been attributed to increased nutrient availability, specifically nitrogen (Radosevich & Conard 1980, Oechel & Hastings 1983, Fleck *et al.* 1995). However, Keith *et al.* (1997) demonstrated how increased phosphorus availability increased wood biomass in a mature snowgum (*Eucalyptus pauciflora*) forest, but also decreased root biomass, resulting in little change in net productivity. Therefore, although ash deposited during fire may partially account for the increase in leaf biomass of *X. preissii*, it is possible that this effect may reflect a change in carbon allocation and not necessarily a net increase in plant productivity.

Despite enhancing biomass production, no evidence was obtained to indicate any role of ash in stimulating the production of new leaves. Stored carbon remobilised for growth following fire (see Chapter 3) contributes to leaf production at the apical meristem. While the apex is receiving carbohydrate from this endogenous source, any effect of an increase in photosynthates exported by maturing leaves under the influence of nutritient enrichment may be diminished.

The effect of ash addition showed greater bias than any distinction between its effect on biomass production versus leaf production. Specifically, ash addition only encouraged new leaf biomass production, implying that after fire nutrients are preferentially directed from the roots to the youngest leaves surrounding the apical meristem, where they act to increase growth. Alternatively, it is possible that nutrient enhanced growth of the pre-existing leaves may have been masked by time and an inherited maximum leaf size.

Within the timeframe of the experiment all pre-existing leaves, regardless of treatment, may have been capable of expanding to a genetically determined size limit, masking any differences in growth rate between pre-existing leaves of grasstrees that received ash and those grasstrees that did not. In contrast, the potential for new leaf biomass accumulation over the same period was limited only by the capacity of photosynthesis and growth rate imposed by treatments.

The positive response of *X. preissii* to ash addition meant that nutrients, most likely released after rainfall, penetrated the soil sufficiently to reach active grasstree roots. Increases in leafblade and leafbase nutrient concentrations in *X. preissii* after fire (Burrows 1998, Wittkuhn 2000, Ward *et al.* 2001) are in keeping with this conclusion. The evidence of nutrient uptake by grasstree roots contradicts the theory that the root systems of resprouting species are too deep to access ash-derived nutrients (Wisheu *et al.* 2000), and favours the idea that resprouters are likely to benefit more from the nutrient-rich postfire soils than their ecological counterparts regenerating from seeds (Biswell 1974, Keeley & Zedler 1978, Grove *et al.* 1986). However, to fully explore these two conflicting hypotheses it would be useful to identify how soon after ash application did the grasstrees show a positive growth response. For such work I would suggest a more powerful experiment (e.g. increased replication) capable of identifying the first, potentially small effects of ash addition.

Shade

Grasstree leaf growth can be reduced by applying shade, consistent with results presented by other researchers for a variety of plants (Hulbert 1988, Gentle & Duggin 1997b, Vila 1997, de Groot & Wein 1999). The response of *X. preissii* suggested a resilience of this species to shade, indicated by relatively weak negative correlations between percentage shade and leaf biomass and leaf number (Fig. 5.11a–c). If considered in relation to an increase in incident radiation following a reduction in overstorey cover due to fire, an increase of PAR by 87% (246 ± 8 μmol m^{-2} s^{-1} incident PAR increased to 1940 ± 6 μmol m^{-2} s^{-1}) induced an increase in leaf biomass of 25% and an increase in the number of new leaves produced by 15%, over the year. This was the largest effect of shade on grasstree leaf grow recorded, and was comparatively quite low compared with findings by Stoneman *et al.* (1995) who measured an eight fold

increase in shoot dry weight for jarrah seedlings at a site where the overstorey had been removed (increasing PAR by only 53%). However, this enhanced growth was the net result of the combined effects of increased PAR, warmer soil temperatures, which were considered to have caused a relatively small component of the growth response (Stoneman & Dell 1993), and improved plant water status, to which growth and photosynthesis rates were most sensitive (Stoneman *et al*. 1994).

Realistically, within its distribution, *X. preissii* is unlikely to experience an 87% increase in PAR as the result of fire removing the overstorey foliage, primarily because the undamaged natural canopy density is never likely to be sufficiently dense. Banksia woodland canopy density at YAB99 was reduced from 49 ± 6% to 30 ± 6% as the result of leaf combustion and subsequent leaf drop due to scorch from the autumn-prescribed burn. While the relationships between percentage shading and leaf biomass and leaf production suggest a possible advantage to grasstrees resprouting in this modified light environment, the associated increase in PAR with this level of canopy opening is likely to account for very little of the typical postfire vigour of *X. preissii*. An autumn wildfire or a crown fire in jarrah forest causing total defoliation (Bell *et al*. 1989) would be likely to induce a stronger light dependent postfire growth response in *X. preissii*, because of the initial canopy cover (73 ± 1%) being significantly greater than in banksia woodland. In either habitat any positive effect of improved light conditions would be short-lived, ensured by the rapid recovery of the overstorey, as demonstrated for the banksia woodland in my study (Fig. 5.12).

Water

During winter, rainfall did not have any value for predicting grasstree growth (Section 2.3.1.1). Consequently the lack of a response to watering over this period may have acted to dilute any positive effect this treatment had during spring and summer. However, not even during autumn when *X. preissii* has been shown to react positively to significant rainfall (see Section 2.3.3.2) was an effect of the watering treatment recorded. Two possible conclusions can be drawn: firstly, that the volume of water applied was less than the equivalent of 18 mm of rainfall that has been observed to induce a leaf production response during summer/autumn, or secondly, that the distribution and application rate of the water, despite considerable efforts, was not

consistent with grasstree rooting patterns. Although an effect of watering was apparent from predawn summer water potentials, it was not large enough to disregard the second conclusion.

Soil water availability around grasstree roots was not enhanced by the watering treatment, evident by the similarity of predawn water potentials between grasstrees not receiving water and those watered (Fig. 5.9a,b). This is consistent with evidence presented in Chapter 3, which also discounts speculation that improved water status supports grasstree growth after fire. Further consistency was revealed by the midday water potential results. Reduced crown size was suggested to be the cause of higher (less negative) midday water potentials in recently burnt grasstrees relative to those unburnt (Chapter 3). This interpretation is supported by the substantial difference in spring midday water potentials between clipped (unwatered) grasstrees from the fire-simulation experiment and unclipped control grasstrees (Fig. 5.9b). This difference was marginal during summer and possibly obscured by site differences indicated by varying predawn water potentials. By autumn, crown regrowth was sufficient to neutralise any effect.

5.4.2 Grasstree resilience to frequent herbivore damage

X. preissii was particularly resilient to the sort of damage inflicted by herbivores that exist within its distribution. Even after total defoliation of the crown, imposed at monthly intervals for 16 months, *X. preissii* demonstrated a strong capacity to recover in both jarrah forest and banksia woodland. The toll of this regenerative effort could be measured as a large reduction in desmium starch reserves: 85% for jarrah forest grasstrees and 88% for banksia woodland grasstrees. This dependence of vegetative regrowth on starch reserves has been identified repeatedly (Donart & Cook 1970, Kosola *et al*. 2001), and starch has been demonstrated to be the most critical resource (Miyanishi & Kellman 1986) to the survival of resprouter species susceptible to defoliation by herbivory and fire (Bamber & Humphreys 1965).

A conspicuous result of this experiment was the stimulation of regrowth due to four-monthly herbivory on grasstree regrowth in both habitats. Particularly for jarrah forest grasstrees, this treatment response demonstrates the level of crown damage sustainable by *X. preissii* during a 16–month period without a negative impact on leaf production,

and arguably the species' general regenerative performance. More specifically, this treatment induced a degree of growth overcompensation (stimulation of growth above that of the controls), most evident in the leaf production response. This result suggested no unfavourable impact of this simulated herbivory regime, yet the costs are subtly disguised as a substantial depletion (89%) in available starch. Therefore, while grasstrees can respond to this frequency of clipping with vigorous regrowth for up to 16 months, further damage at this rate, completing the depletion of reserve starch, could retard further growth of these grasstrees or even jeopardise their survival. This likely scenario reflects the conclusions by Lindroth (1989) regarding plants that show overcompensatory growth following herbivory. However, his generalisation that growth overcompensation is characteristic of plants growing on nutrient-rich sites does not hold true for *X. preissii,* which grows on infertile soils throughout its distribution (Hobbs *et al*. 1995a). It is easy to see that without the consideration of reserves, the cost of herbivory on *X. preissii* may be inaccurately estimated or go totally unnoticed. Similarly, Silvertown and Dodd (1999) discussed the importance of assessing reserves to avoid the underestimation of costs associated with reproduction in perennial plants.

Despite the response just described, herbivory frequency was generally negatively correlated with grasstree growth performance in jarrah forest, which in turn was ultimately a function of reserve starch availability: the more frequently a plant was damaged the more starch it demanded. Starch reserves did not appear to limit grasstree leaf production in banksia woodland under any of the herbivory frequencies, as foliage clipping consistently produced a stimulatory effect beyond that of normal growth. In the same habitat the two lowest frequency treatments stimulated grasstree biomass production, as described for leaf production, but the highest herbivory frequencies (every one and two months) failed to induce a similar response in leaf biomass production. The disparity between these results implies that even when starch reserves are dwindling they are still used indiscriminately within the crown. It may be predicted that if starch reserves were rapidly running out they would be best spent in developing current young leaves to a condition where they may contribute to carbon demands. However, in a depleted state reality showed that remobilised carbohydrates continued to support the production of new leaves, despite the negative impact of low starch reserves on the elongation and expansion (biomass production) of leaves reaching photosynthetic maturity. An investigation of the partitioning of carbon between the roots and shoots

during a period of herbivory would be of interest. While a study of three annual species demonstrated that when nutrient availability changed no adjustment to the root/shoot partitioning of biomass occurred (McConnaughtay & Coleman 1998), *Eucalyptus pauciflora* responded to similar changes with a considerable shift in carbon allocation (Keith *et al.* 1997). These studies could be considered to parallel the changes in growth and resource availability in *X. preissii* during herbivory, but clearly the stimuli are quite different. I predict that as a survival mechanism for *X. preissii,* root growth would decrease or cease altogether, even during light herbivory, as nutrients and more importantly carbon are preferentially translocated to the rapid leaf growth above ground.

The greater resprouting performance of banksia woodland grasstrees than those in jarrah forest points towards a likely difference in resource availability that favours growth on the Swan Coastal Plain relative to the Darling Range. However, comparisons of the size of grasstrees at the two sites have not revealed consistent differences (contrast results presented in Section 3.2.2.1 with those from this experiment), therefore failing to offer support for this argument. The 19% greater mean starch index (relative starch availability) in unclipped control grasstrees from banksia woodland was convincing evidence of the availability of larger carbon reserves in those plants. This may reflect the marginally larger average stem circumference of banksia woodland grasstrees (84.8 ± 1.6 cm) than for the jarrah forest plants (70.8 ± 1.3 cm). Such a difference in starch reserve size may account for the variability of responses between grasstrees from each habitat, and would help to postpone the symptoms of chronic herbivory in the banksia woodland plants.

Port Lincoln Ringneck parrots cause severe damage to remnant stands of *X. preissii* in Western Australia's intensively farmed wheatbelt region, particularly when agricultural crops (the preferred food source) have died off in early summer and green feed is limited (McNee 1997). As in this case, pressure from herbivores can often be exacerbated by dry conditions (Zamora *et al.* 2001). Parrots are capable of totally defoliating a grasstree crown, and from anecdotal evidence McNee (1997) suggests that grasstrees may persist for 4–7 years before death results. Because of distinct differences between this scenario and the regime of foliage damage described during this study (e.g. seasonal herbivory versus continuous herbivory), it is hard to speculate on the likely frequency of parrot visits to wheatbelt grasstrees and the role that carbohydrate reserve depletion plays in the

death of these plants. However, from the information at hand the likely sequence of events that leads to the demise of *X. preissii* as the result of parrot herbivory, or similar chronic herbivory can be inferred. It is clear that *X. preissii* starch reserves are sufficient to support new crown growth after an initial season of browsing imposed by parrots during summer/autumn, despite its coincidence with minimum annual starch reserves in late autumn (Section 3.3.4). It has been demonstrated that the impact of grazing is greater when starch reserves are at an annual low in grass and shrub species in montane Utah, compared with when they are at an annual high (Donart & Cook 1970). If this pattern of impact is consistent for grasstrees, the effect of regrowth costs may be compounded.

No effect of applied clippings was apparent during this study, possibly as strong resprouting by *X. preissii* acted to rapidly push developing leaves up through the clippings, preventing shading and smothering effects. Similarly, leaf growth rates between periods of intense parrot browsing may be initially high, and new leaf elongation may aid in the dissipation of leaf remnants and circulation of air around the apex. While the winter months offer a period for recovery of damaged grasstrees (lowest annual browsing pressure (McNee 1997)), the minimum temperatures are not conducive for leaf growth (Chapter 2), but may allow photosynthate production (Larcher 2000) that contributes to the replenishment of desmium starch. If the same grasstrees are repeatedly damaged over successive summers, the net effect is likely to be a gradual depletion of starch reserves. Following shoot destruction after burning, starch reserves of a mediterranean shrub (Bowen & Pate 1993) and two neotropical savanna shrubs (Miyanishi & Kellman 1986) took up to 1.5–2 years to return to pre-burn levels: considerably longer than the 6–7 month reprieve of *X. preissii* from parrot browsing. After several years crown recovery would become negligible, and residual leaf would begin to persist about the apex, creating an environment conducive to rot, encouraging death.

5.4.3 Is rapid leaf growth response to crown damage of *X. preissii* an adaptation to fire or herbivory?

Repeatedly, data presented in my thesis has revealed a significant stimulatory effect of foliage removal on the subsequent growth of *X. preissii*, particularly evident in this chapter.

1) Grasstrees demonstrated a rapid flush of leaf production after spring or autumn fire consumed much of the existing crown.
2) Removal of all foliage following flowering, a nutritionally and energetically stressful period, stimulated a rapid and temporarily sustained flush of leaves.
3) Clipping of the entire crown to simulate the damage caused by fire was shown to increase leaf production, and
4) Complete removal of all foliage in unburnt jarrah forest, at varying frequencies, again confirmed this species' plastic responses to crown damage.

Without prior knowledge of Australia's pre-history, and in the context of the present vulnerability of the Australian bush to fire, the obvious conclusion to draw from an examination of grasstrees morphology, flammability and early seedling establishment is one of adaptation to its fire-prone environment (for detail see the Introduction of Chapter 3). Logically, it follows that the postfire growth response is simply another example of such adaptation. Yet, increasingly, data collected during my study suggest that this may not necessarily be the case.

The natural occurrence of fire is a relatively new factor influencing Australia's flora, as its frequency has only recently increased with the continent's gradual shift to aridity during the late Tertiary (Hopper *et al*. 1996), and possibly within the last three million years (Specht 1973, Specht & Specht 1999). Flannery (1994) suggested that the importance of the role of fire in today's landscape reflects its replacement of the long extinct herbivore megafauna that roamed much of Australia: the standing biomass once recycled as faecal pellets, now more commonly is returned to the soil as ash. While the exact interchangeable nature of the megafauna and fire in this particular role may be questionable, the chronology of the relative importance of herbivory predating fire seems quite certain.

Evidence is more often presented for the timing of the disappearance of Australia's megafauna from the fossil record (Archer 1984, Dawson & Augee 1997) than their appearance, but it is generally agreed that faunal gigantism prevailed from the Pliocene (starting 5–7 million yrs BP) to the Pleistocene epochs (Hopper *et al*. 1996, Augee & Fox 2000). However, Australia's populations of herbivores were well established by this stage, with the diprotodonts (one of the two orders of extant marsupials, the majority of which are herbivorous) emerging as early as 63 million years ago (Woodburne 1996), and no later than the Eocene (from Hopper *et al*. 1996). Specific to the current plight of remnant *X. preissii* populations in the wheatbelt region of Western Australia, the global fossil record of parrots is generally scant, let alone specific to Australia, as their bones are not predisposed to fossilisation, which is also hindered by the lack of water in their habitat (Forshaw 1978, Rich & Van Tets 1984). While the oldest fossil parrot (*Archaeopsittacus verreauxi*) was unearthed in France and dated at about 30 million years (Forshaw 1978), predictions of the appearance of parrots in the Australian landscape have suggested the mid to late-Cainozoic (from 5–20 million years ago. Rich & Van Tets 1984). Although, this is a very broad estimate it does suggest that parrots may have been feeding on grasstrees for some time.

Native fauna, such as the kangaroo and wallaby, are considered to exert minimum grazing pressure on the plant communities of Australia (Specht 1973). But the array of mechanical and chemical herbivore deterrents that are exhibited by the present Australian flora imply that this may not have always been the case. The high incidence of spininess of southwestern Australian heath species has been suggested to be a defence mechanism against browsing megafauna with the ability to survive on plant material of very low nutritive value (Grubb 1992, Hobbs *et al*. 1995a). Other mechanisms with contemporary relevance to plant survival imply more than a minimum impact of modern herbivores. For example, 'self-crypsis' shown by *Hakea trifurcata*, where fruits mimick the unrewarding broad leaves reducing herbivory by white-tailed black cockatoos (*Calyptorhynchus funereus latirostris*. Groom *et al*. 1994a).

From this perspective it is hard not to wonder whether the growth response of *X. preissii* to fire could in fact represent an adaptation to sustained grazing pressure over millions of years, and the responses observed during this study reflect the similarity between the action of fire and herbivory on this species: a level of stress accompanied by substantial

crown damage. Artificial removal of leaves more closely approximates the damage caused by browsing animals than the effect of fire yet causes a similar response. Flowering of *X. australis* has been shown to be stimulated by simply clipping the foliage (Gill & Ingwersen 1976), and although the probability of plants flowering may be improved by burning, perhaps the application of a Diprotodon-sized dropping beneath a grasstree would help to achieve a comparative response!

To take this line of reasoning a step further, the grasstree characteristics attributed to fire resistance can be viewed as being derived from selective pressures driven by herbivory. In the absence of a protective layer of bark to deter browsing and boring herbivores from harvesting the nutrient-rich inner stem, *Xanthorrhoea* spp. have compensated by retaining their dead leaves and impregnating them with a hard, indigestible resin. With large enough herbivore populations the gradual accumulation of dead skirt would be regularly trimmed, preventing the rot, termite damage and stem breakage (due to excessive thatch weight) seen today in long unburnt stands (Ward 2000). The significance of the flammable skirt may represent mere coincidence, a conclusion difficult to accept under contemporary natural fire frequency, but more likely in a damper, subtropical Australia of 65 million years ago. The dense packing of green foliage around the apex seems like a common solution to an organisational problem of many leaves growing from a single meristem, a feature shared by related plants from other habitats, including palms, cycads, *Dracaena* spp. and *Yucca* spp. For grasstrees, this arrangement affords maximum protection of the most palatable foliage by the radially orientated spear like leaves. Also, perhaps the contractile roots of grasstree seedlings (Staff & Waterhouse 1981) were selected to draw the defenceless young plant belowground, avoiding irreparable damage caused by browsing vertebrates. How quickly would we have correlated the biology of *X. preissii* with fire adaptations if this species only currently existed under more mesic subtropical circumstances regularly grazed by populations of large herbivores and where fires were rare?

CHAPTER 6

Synthesis

Testament to the broad range of climatic conditions experienced during one annual cycle within mediterranean Australia is the partitioning of most plant species into groups with well-defined growing seasons, often sharing similar growth form (Specht *et al.* 1981, Bell & Stephens 1984, Lamont & Bergl 1991, Hobbs *et al.* 1995a). *X. preissii* is an exception, growing continuously, but with a fluctuating pattern reflecting seasonal changes in temperature and rainfall (Chapter 2, and see Fig. 6.1 for a summary). As it is able to function under a wide range of temperatures and respond rapidly to bouts of rain, *X. preissii* is able to achieve uninterrupted growth. While the capacity to grow during the heat of summer categorises *X. preissii* as a mesotherm (having a subtropical growth rhythm), its ability to sustain growth at winter temperatures as low as 10 ˚C contradicts limits of the term (Specht & Specht 1999) and overlaps with the expected thermal range of microtherms (warm-temperate). The range of field temperatures recorded during leaf production monitoring was too narrow to reach the thermal optimum of *X. preissii*, emphasising the apparent mismatch between its physiological temperature range and its current environmental temperature range.

Positive responses to high temperatures are considered a relic of the warmer Gondwanan period (Specht & Dettmann 1995, Specht & Specht 1995, Specht & Specht 1999). Despite retention of such ancestral features, grasstrees have also evolved with their changing environment, especially lower temperatures and water availability. In southwestern Australia, low temperatures in winter (despite some adaptation to these) and drought in summer generally restricts growth. Consequently, with increasing aridity in the late Tertiary (Kemp 1981, Specht & Dettmann 1995, Hopper *et al.* 1996, Kershaw *et al.* 2002) the distribution of grasstrees would have receded into the damper, peripheral parts of the continent, under selective pressure to adapt towards greater drought resistance. Synchronisation of leaf death with summer drought, and the ability to harvest temporary surface water from episodic summer rainfall (Chapter 2) are two attributes that contribute to this ability. It is likely that grasstree leaf growth, unrestricted by soil moisture and low temperatures under the climate of Tertiary Australia, would have

exceeded current rates, and the next most limiting factor (nutrient availability?) would have dictated the maximum growth rate.

Fire marks the start of a dynamic phase of grasstree biology, commencing with rapid sprouting (Chapter 3, and Fig. 6.1), primarily due to the 'clipping' effect of fire. The simplicity of this mechanism contrasts with the potential complexity of its vast array of exogenous and endogenous implications. Possible physiological effects include exposure of the inner crown to increased light (photosynthesis) and temperature (respiration), reduced diurnal ψ_x, and hydrolysis of reserve starch into soluble sugars. Secondary to this effect, increased ash (nutrient) availability, reduced overtopping by larger species, and subsequent weather conducive for growth, might contribute to the quick restoration of the fire-damaged crown (Chapters 2 and 5). Leaf removal, simulating the action of browsing herbivores, was capable of inducing a similar response, which questions the evolutionary significance of such rapid growth (Chapter 5). Are grasstrees specifically adapted to recovering after fire, or is a long history of herbivory pressure responsible for pre-adapting grasstrees to respond to leaf removal?

Compensating for reduced photosynthetic potential after the loss of foliage, starch stored in the stem is remobilised and consumed during the resprouting process. Minor annual fluctuations in the starch reserve reinforced the conclusion that the starch is reserved for periods of particularly rapid growth, whether vegetative (Chapter 3) or reproductive (Chapter 4). Peak starch stores in summer may suggest a strategy to coincide with the time of greatest fire risk, but this period also represents a time when tender foliage is scarce and *X. preissii* is most prone to herbivore damage (McNee 1997).

While still undergoing a fire-induced, vegetative growth response (Chapter 3), *X. preissii* commences reproduction, producing a flowering spike within three months that commonly reaches over 3 m long and weighs several kilograms. Not surprisingly trade-offs occur to accomplish this feat. Carbohydrate requirements are primarily met by the redirection of photosynthates to the growing inflorescence, sacrificing leaf production as a consequence. Secondary to this source, starch reserves are substantially depleted and the photosynthetic inflorescence also ameliorates the cost of its own construction (Chapter 4, and see Fig. 6.1 for a summary).

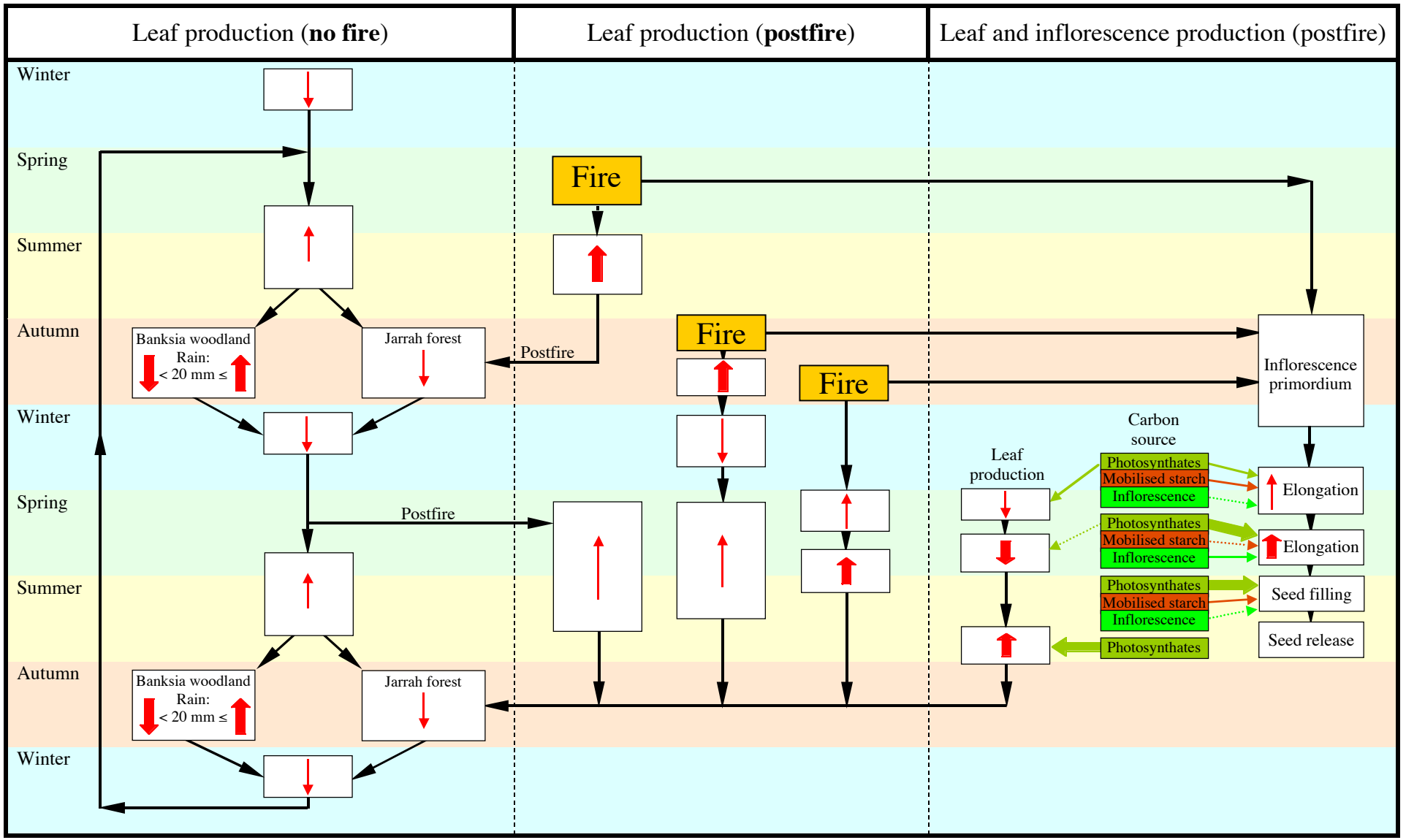

Fig. 6.1 Summary of the effect of fire and season on grasstree growth in jarrah forest and banksia woodland, and the contribution of carbon from three sources to both vegetative and reproductive growth during each stage of reproduction. Red arrows represent the relative rate of leaf production or inflorescence elongation (⬆ = rapid increase, ⬇ = rapid decrease, ↑ = gradual increase, and ↓ = gradual decrease). The relative amount of carbon each source contributes to growth during each stage of reproduction is indicated by the size of the respective arrows (➡ = high, → = medium, ⇢ = low).

6.1 Relevance of the results to the banding technique

Plants are subjected to an ever-changing set of environmental variables to which they react. Some physiological responses occur within minutes of the event (Fritts 1976). While such responses may be scarcely detectable, the integration of a continuous stream of stimuli-responses is expressed in a complex way as measurable growth. Growth of tree rings has been recognised as a source of environmental information since as early as the 15th century, when Leonardo da Vinci first noted the relationship between tree-rings and precipitation (Stallings 1937, from Schweingruber 1988). The application of dendrochronological techniques is often met with considerable limitations; factors such as species, location, elevation, topography, light, conservation issues and interpretation all play a role in the accuracy and value of results, and the cost of their collection on the environment (Schweingruber 1988, Burrows *et al.* 1995, DesRochers & Gagnon 1997, February & Stock 1998a, February & Stock 1998b). Such techniques have also incorporated the reconstruction of fire history using fire scar analysis, but again, success can vary and can be particularly limited for identifying low intensity fires (Burrows *et al.* 1995, Niklasson & Drakenberg 2001).

The leafbase banding technique (Ward *et al.* 2001) is a unique form of dendrochronology, with specific importance for reconstructing fire history. Its distinctiveness relates to its use of the regular banding pattern on the stem of arborescent monocotyledons (grasstrees), rather than the traditional concentric growth rings measured in conventional dendrochronological studies of dicotyledonous trees and conifers. As demonstrated in Chapter 2, one year of leaf production by *X. preissii* equalled one pair of adjacent cream and brown bands, representing the manifestation of continual growth responses to an annual climatic cycle characterised by two distinct seasons. The following sections discuss the further significance of my research in respect to the banding technique.

6.1.1 Seasonality of band colour

The colour difference between the alternating seasonal bands is difficult to explain. The bimodal pattern of grasstree growth conveniently suggests the timing of this dichotomy (Chapter 2), although the mechanism that controls leafbase colour is unclear. Colangelo

the colour difference between the cream and brown leafbases, but could only speculate on a possible reason for the disparity. Leaf initiation and elongation respond similarly to favourable growing conditions, both peaking between late spring and early autumn when water is not limiting. In contrast, from the end of autumn and through winter, unfavourable growing conditions restrict this kind of rapid leaf initiation and vigorous leaf expansion.

Tannins commonly accumulate in plant leaf tissue in response to adversities in their environment, protecting the plant against rotting as a result of bacterial or fungal action, dehydration or damage by animals (Fahn 1990). The poor growing conditions starting in late autumn may be responsible for the higher level of tannins in the associated leaf growth, or tannin accumulation may be a response to an increase in the frequency of detrimental events linked with winter. For example, *X. preissii* is susceptible to a number of pathogens, including *Phytophthora cinnamomi* (Shearer & Dillon 1995) and the leaf spot, *Colletotrichum xanthorrhoeae* (Shivas *et al*. 1998), that are more abundant in wet conditions. The noticeably more common occurrence of *C. xanthorrhoeae* on the wetter coastal plain than further inland emphasises this pathogen's infectious behaviour under highly humid conditions characteristic of winter, where dispersal is by rain-splash (Shivas *et al*. 1998).

The higher bulk density of slow growing leaves (D. Korczynskyj, unpublished) and thinner bases (Eldridge 2000) may concentrate the available tannins in the winter formed leaves, effectively darkening leafbase colour. Accumulation of any substance including tannins implies a function of time. Leaf age at maturity is dependent on the season of initiation. Leaves produced during summer mature earlier than leaves produced in other seasons, suggesting a shorter lifespan. Since leaf death occurs exclusively in summer and leaf age at death is about two years, the lifespan of a leaf formed in June during winter would be about 2.5 years compared with a leaf formed in November or December that would die after the minimum two years. Leaf longevity could therefore also explain differences in leaf tannin abundance. An histological investigation of living leaves may reinforce the evidence for the tannin-based origin of leafbase colour differences. By examining a series of different aged leaves it may be possible to correlate time with tannin concentration.

6.1.2 Fire effects and banding patterns

Except for the grasstrees burnt in late autumn, all other grasstrees in my study exhibited rapid leaf production and elongation almost immediately following fire. From what is understood of the pattern of seasonal leafbase banding (cream and brown bands) it would be expected that this fast growth would be recorded as a wider band containing considerably more leafbases than subsequent seasonal bands and possibly thicker leafbases, particularly if the lower bulk density of fast growing leaves (D. Korczynskyj, unpublished) reflects larger leaf dimensions. Surprisingly, in a study of stem banding patterns, little evidence of wider postfire bands was obtained (Eldridge 2000). The only evidence of a postfire growth flush was a trend for leafbases of the seasonal bands produced after fire to be thicker than those produced immediately prior to fire.

The absence of an increase in band-width and leafbase number following fire is difficult to explain given the dramatic effect of fire on leaf production and elongation. A possible reason may be the reduction in leaf production as a consequence of inflorescence elongation (see Chapter 4) that would act to reduce band-width and leafbase numbers. This may account for why the only evidence of a positive effect of fire was from leafbase thickness. The probability that the grasstrees used in the study by Eldridge (2000) flowered routinely after fire is increased by virtue of their height (mean height = 173 cm), as tall grasstrees are more likely to flower than shorter ones (Ward & Lamont 2000). Furthermore, it is likely that these plants would have been burnt more often during the hot/dry summer and autumn months when fire is more prevalent, which has also been shown to increase the chances of this species flowering (Lamont *et al*. 2000). It therefore seems reasonable to assume that following fire, the grasstree specimens used by Eldridge (2000) may have flowered more often than not, severely reducing the chances of demonstrating an effect of fire on the banding pattern.

To resolve this confusion it would be useful to apply the leafbase banding technique to the grasstrees studied during the work described in Chapter 3. I made such an attempt. However, as many of the tagged leaves were still living or partially green, the record of banding was not distinguishable. A second attempt should be made once the tagged leaves have completely senesced. The information gathered would not only provide a direct correlation between leaves of known growth history and stem banding patterns,

band. This work would be particularly valuable because of the spatial and temporal replication incorporated into the original design of the study.

6.2 Extension of the leafbase banding technique and implications for further research on grasstrees

6.2.1 The leafbase banding technique

The follow-up work described above represents only one opportunity for further research into developing the leafbase banding technique. As a new tool for gathering historical data about grasstree age and fire frequency the technique may still be considered in its infancy, especially on a national scale where its potential is under–explored. With further application and critical analysis of the data, using the understanding of grasstree growth developed during my study, the technique could potentially recognise other environmental pressures such as herbivory and canopy opening (in jarrah forest or banksia woodland for example, Chapter 5) caused by the pathogenic fungus, *Phytophthora cinnamomi* (dieback. Dell & Malajczuk 1989), therefore broadening its use. Changes in seasonal band-thickness, or number and/or size of leafbases per band, may offer clues to the past growing conditions imposed by the environment. Warmer/wetter conditions, particularly during summer, would support greater leafbase production than cooler/drier conditions. A seasonal band containing significantly more leafbases, followed by successive bands with progressively less leafbases, may identify a period of chronic herbivory. The application of the banding technique to the grasstrees used for the herbivory study in Chapter 5 (after clipped leaves have senesced) would provide a starting point for this line of investigation. Identifying and interpreting the chronology of dieback infection may prove more challenging, as although overstorey crown death allows greater light penetration and stimulating grasstree leaf growth, grasstrees themselves are susceptible to the pathogen (Dawson *et al*. 1985, Shearer & Dillon 1995). Specific details of the effect of dieback on grasstree leaf growth could benefit the possible use of the leafbase banding technique for this purpose.

In addition to deciphering information from inter-fire periods, preliminary observations indicate that the thickness and uniformity of black fire bands around the stem

fire (D. Ward, CALM research, pers. comm.). Such information regarding fire conditions are commonly recorded during current prescribed burning practices and would provide a sound basis for a correlative study.

6.2.2 Other areas requiring further research

A typical consequence of investigation is the emergence of new questions, and my research was no exception. Some directions for further study were suggested during the previous four chapters and need little explanation. For example, protein in and adjacent to the desmium tissue may play a similar role in *X. preissii* to that of starch, and is worth pursuing. Below, I have clarified some of the previously mentioned areas of future research, in addition to providing others.

In banksia woodland, grasstrees resisted water stress and responded rapidly to single large rainfall events during summer/autumn drought, consistent with having live, functioning roots in the deep and shallow soil horizons. Hydraulic conductance was suggested as a means of maintaining root/soil contact during summer while avoiding desiccation, as described for *Banksia prionotes* in the Swan Coastal Plain (Pate *et al.* 1998), and is an area that needs further investigation. Research demonstrating summer water status of grasstrees (Crombie *et al.* 1988, Crombie 1992, this thesis) has been based on relatively large representative plants (grasstrees about 0.8–1.2 m high, potentially 50–100 years old). It would be of interest to conduct a comparative study on small acaulescent grasstrees that have not established a root system of mature depth. This work may give some insight into the relative importance of rooting depth to continuous leaf growth through summer, and if conducted in banksia woodland, could also indicate whether the size of the root system is important for the exploitation of episodic summer rainfall. Specifically, is the root system large enough to gather sufficient water, and do vertical roots penetrate deep enough to attain water for hydraulic conductance to sustain shallow lateral roots?

The timeframe within which *X. preissii* re-establishes a crown, flowers and releases seeds following fire is relatively short, and coincides with the duration of the fire effect on this species (Chapters 3 and 4). The timing of these events appears to be important for reproductive fitness. However, assessing fitness of slow growing species is impractical and indicators of fitness (reproductive effort, seedling establishment, growth

and survivorship) could be explored relative to increasing time since fire. If 'reproductive fitness' was improved by shortening the period between fire and reproduction, autumn-burnt grasstrees (flowering in the following spring) would have an advantage over spring-burnt grasstrees. However, the time available for crown recovery is reduced following autumn burning, with possible implications for reproductive fitness (a smaller inflorescence was produced, Chapter 4). Therefore, a working hypothesis should state that summer burns (a compromise between the timing of fire seasons) are more beneficial to grasstree reproductive fitness than autumn or spring burns. Tentatively, this hypothesis is supported by greater fruit production by summer-burnt grasstrees (Lamont *et al*. 2000).

Grasstrees provide a habitat, food and refuge from fire for many invertebrates and vertebrates (Whelan 1995), which raises many question regarding plant-animal interactions. During this study, the voracious feeding habit of moth larvae (*Meyriccia latro*) proved to be a significant threat to the success of my fieldwork on grasstree reproduction. Their ability to consume and kill the developing inflorescence questions the sustainability of this relationship: can grasstree populations recruit sufficient seedlings despite these parasites to compensate for grasstree death rates? Furthermore, as inflorescences are often killed early in their development, and larvae were not noticed on vegetative grasstrees, does this moth target reproductive grasstrees only? And if so, what cues does the moth use to identify these individuals? Visual inspection prior to obvious spike emergence was unsuccessful in recognising reproductive grasstrees during my work and that of others (Staff 1976).

Finally, the work documented in this thesis, as part of a collaborative effort by the Curtin University Grasstree Project Group, represents the most comprehensive study of any of the Australian grasstree species. This strong background of information provides the opportunity for other comparative studies on grasstrees in other climate regions of Australia. For example, it would be of interest to investigate the water relations of the only arid zone grasstree species, *X. thorntonii*, that grows as far inland as the Great Victoria Desert, north of the Nullarbor Plain (Kealley 1993). Furthermore, how does this species distribution affect its growth phenology, and does water availability play a role in the ability of these grasstrees to reproduce in response to fire? Temperature and soil moisture responses of this species may be useful in furthering our understanding of

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